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The impact of cultivation techniques on bioactive compounds in the aerial parts of *Echinacea purpurea*

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Sm08

A NEW LOOK AT MEDICINAL AND AROMATIC PLANTS

Sm08.001

A New Look at Medicinal and Aromatic Plants

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Herbs, Medicinal and Aromatic Plants (MAPs) have been utilized in various forms, since the very early periods of mankind. Even until recently, these resources were exploited nearly without any major limitations. The initial basic curative role of MAPs has been maintained, since even today 80 % of the world's population relies primarily on traditional medicines. In the developed parts of the world, however, an opposite new trend has emerged, i.e.: To seek natural alternatives to using conventional drugs, food and cosmetics, etc. with lesser side effects. As a counterpoint to their basic traditional culinary, as well as food industry uses to-date MAPs are intensively consumed in the form of food supplements and food additives. More recently feed-additives have gained on popularity. They offer natural alternatives with less side effects to animal and ultimately to human consumption, instead of the synthetic chemicals and production increasing hormones, formerly used in animal husbandry. All these, and farther, here not detailed factors, seem to maintain a varying and ever increasing huge demand on these commodities of already limited availability. A New Look, a different holistic focus and R+D action is needed to improve and sustain a vibrant and socio-economically sound MAPs sector. Main elements of this can be best related to the sources of raw material production: The primary resource for raw materials is wild-crafting with some over nine tenth of MAPs used traditionally gathered in the so called Third World countries. The huge demand has already lead to the overexploitation of natural resources, thus also endangering valuable incomes for rural households, especially in developing countries. Consequently, the sustainable use of natural resources has become an inevitable imperative from both environment protection and socio-economical points of view. Farther efforts should be made to secure a steady stream of raw material supply of these important species by *in situ* and/or *ex situ* production technologies. In addition to conserving and farther improving the germplasm of traditionally cultivated species, the domestication/introduction of farther crops is needed. Sophisticated *in vitro* micropropagation and breeding (selection) technologies aided by advanced phytochemical and molecular biological analytical techniques can farther assist this process. Ultimate goal of these efforts should be to assist the recent upsurge in improving the traceability and safety (reliability) of natural products, as well as the appropriate policies and legal frameworks to guide the protection, production (including organic production), trade, and applications of medicinal and aromatic plant materials (GWP, GAP, GCP, GMP, etc.).

Sm08.002

Hybrid Condiment Paprika Breeding and Adaptation of Agrotechnical Methodology in Hungary

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The authors would resolve the future of the condiment paprika production – at least partly - with the forcing house production of new, Hungarian bred hybrids.

For this purpose the authors: - bred and announced for registration three new condiment paprika hybrids, - worked out the production technology of shoot regeneration for condiment pepper hybrids, adapted successfully the applied methods of shoot regeneration for sweet pepper production, - defined the fundamental correlation between the mode of cultivation and the yield quantity and the nutritional value, - proved that during the development process the tested lines were significantly different in yield quantity and quality, and these features are mainly determined by their genetic background, - based on their observation they are supporting the fact that to breed such hybrids, which can be effectively produced, it is suggested to produce the parental lines from European varieties, - proved that the breeding process of the Hungarian condiment paprika can be successfully and cost-effectively speeded up by exploiting the seasonal variations in the environmental parameters. In the breeding phase of the experiment 41 local and foreign lines were used in the crossings and during the four-year-long line surveys around 800 combinations were evaluated based on many thousands of samples. In the course of the agrotechnical experiments they examined the effect of the supporting system, the type of pruning, and the time of harvesting on the quantity of yield as well as on the nutritional values. Based on the results they formulated a recommendation for the producers, who were crucial participants of the breeding work.

Sm08.003

Selection of Sponge Gourd Genotypes High in Productivity and Identification of Anti-Microbial and Anti-Oxidant Activity Characteristics

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Sponge gourd (*Luffa ssp.*) plant is a potent material to be an effective health care food due to its natural characteristics of anti-inflammatory activity. As an initial step of breeding new genotypes high in productivity of sap exudates and biomass, total 32 genetic resources collected from local and overseas were screened. Twenty-five of them are included in the species of *Luffa cylindrica* and five of them are included in the species of *Luffa acutangula*. There was a high genotypic variation in productivity of sap exudates, which had a negative correlation with productivity of biomass in the genotypes of SG2, SG5 and SG24. Several genotypes introduced from overseas, including SG1, SG11, SG14, SG17, SG19, SG21 and SG26, had higher in productivity of sap exudates and biomass than local ones. The worst productivity of sap exudates was observed in the genotypes of SG3, SG8, SG25, SG27 and SG30. The antimicrobial and anti-oxidant activities were analyzed in various plant parts, including sap exudates of stem (basal 50 cm), fresh plant tissues (stem, leaf and root), flowers, fruit flesh, fruit rind, and seed of one local variety. A significantly higher antimicrobial activity against *Malassezia furfur* was observed in the root tissue and sap exudates, while the activity against *Candida albicans* was higher in the leaf tissue and sap exudates compared to other parts. When the activities of antioxidant enzymes, including ascorbate peroxidase (APX), catalase (CAT) and superoxide dismutase activity (SOD), were compared, significantly lower activities were observed in stem tissue than other parts.

Sm08.004

Nitrogen Fertiliser Requirements for South African Medicinal Plant Species Used in Traditional Healing Practices

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The question of whether South African medicinal plants can be cultivated to meet rising demand for medicinal plants in traditional healing practices is a burning

matter. The demand for medicinal plants is related to the great cultural significance attached to medicinal plants. The growing demand has not only resulted in increased hazard for overexploitation of wild populations, but also an increased interest in cultivation. The intensive harvesting of medicinal plants due to increased use has in many places resulted in overexploitation and forms a serious threat to biodiversity. This results in acute shortages and price increases for certain plant species. Very little information is available on growing and cultivation of South African medicinal plants. The effect of nitrogen fertiliser on the yield, chemical composition and antibacterial activity have been determined for three medicinal plant species used in traditional healing practices in South Africa namely *Artemisia afra*, *Leonotis leonurus* and *Sutherlandia frutescens*. Different sources of nitrogen (ureum, lime ammonium nitrate and ammonium sulphate) and different levels of fertiliser (0, 180, 240 300 and 360 kg N·ha⁻¹) were applied as treatments. The fresh plant material was weighed and subjected to chromatographic analysis and bioassays. The test organisms which were used in the bioassay were *Staphylococcus aureus*, *Escherichia coli* and *Enterococcus faecalis*. All the species reacted positively with addition to the three nitrogen sources with most of the treatments showing a significant increase in the fresh mass yield with the lowest level of fertiliser applied. *Leonotis leonurus* and *Artemisia afra* achieved the highest yield at 180 kg N·ha⁻¹ ammonium sulphate and *Sutherlandia frutescens* at 180 kg N·ha⁻¹ ureum. The chromatographic analysis confirmed that no major changes occurred in the plant during the duration of the trials.

Sm08.005

Variability Agronomic, Chemical and Essencial Oil Yield of Cultivars Garlic (*Allium sativum* L.) of the State of São Paulo, Brazil

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The therapeutic activity of garlic is too extensive and can combat cardiovascular endocrine diseases, acting as anti-neoplastic and also has antimicrobial, anti-oxidants and immune system. The pharmacological effect of garlic has been attributed to sulfur compounds obtained by the degradation of allicin, abundant in the tissues of the plant. The state of São Paulo has some of garlic cultivars and the IAC through germplasm bank has been developing new varieties and more productive with better medicines. Based on a study of the IAC itself that led to the classification of 72 cultivars, using the technique of gel electrophoresis associated with morphological characterization, it was the 20 distinct groups, yet the chemical characteristics at the time were not considered. This work aims to characterize the volatile fraction of 16 varieties among groups of cultivars based on sulfur compounds which attribute medicinal properties to the plant in order to identify the best cultivars based on qualitative and quantitative aspects of the main active ingredients present the characteristics associated traits. The extraction of essential oils was made by hydrodistillation. The chemical analysis was conducted on a gas chromatograph coupled to mass spectroscopy, identification of chemical constituents was performed using the comparative analysis of mass spectra of substances with the database of the GC-MS and retention index. The results were submitted to analysis of variance and means compared by Tukey test. To perform the analysis, we considered each individual treatment, with 3 replicates of injection and each chemical was considered a variable to the already adopted agronomic statistically the Scott-Knott. It was observed that the yield and relative proportions of principals' components for the 16 varieties showed difference. The yield ranged from 0.77 to 6.24%, while the relative proportion of disulfide and trisulfide, ranged from 19.62 to 55.27% and 33.55 to 72.48%, respectively.

Sm08.006

Intercropping Studies and Integrated Nutrient Management in Ashwagandha (*Withania somnifera* Dunal.)

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Ashwagandha (*Withania somnifera* Dunal.) belonging to the family *Solanaceae*, is an important medicinal crop cultivated in India under rainfed situations. Roots prescribed for hiccup, bronchitis, rheumatism, dropsy and curing general sexual weakness in human. Intercropping of ashwagandha with pigeon pea (*Cajanus cajan*) revealed that significantly highest gross and net returns of Rs. 35,625 and Rs. 21,625 per hectare respectively, were obtained in intercropping system (Two rows Ashwagandha and one row pigeon pea) followed by sole crop of ashwagandha. The lowest returns were recorded in sole pigeon pea. The highest B:C ratio of 2.54 could be recorded in intercropping system with good quality roots. The total alkaloid content in the roots was not affected by intercropping system. Significantly maximum plant height of 66.34 cm was recorded as the crop was supplied with both organic and inorganic sources of fertilizers (2.5 t FYM + 0.5 t vermicompost + 30:40:30 kg NPK/ha). Significantly highest number of branches was recorded as the crop was supplied with 2.5 t FYM + 1.0 t vermicompost and 30:40:30 kg NPK/ha. The root length was not influenced by both organic and inorganic sources, but root diameter was influenced by the inorganic fertilizer application. However, significantly higher root yield of 1170 kg/ha was obtained as the crop was supplied with 5.0 t FYM + 1 t vermicompost + 30:40:30 kg NPK/ha. Application of farm yard manure (5 t/ha) had significant effect on total alkaloid content (0.204%). Combined application of N, P₂O₅ and K₂O had negative effects on total alkaloid content in the roots. The study included problems faced by the ashwagandha growers, which was collected with the help of personal interview techniques using well-prepared schedule. High cost of seeds, poor germination, and lack of technical information on processing and unorganised market was identified as main problems by the growers.

Sm08.007

The Effect of Variety and Row Distance on Yield and Quality of Pot Marigold

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Calendula or pot marigold (*Calendula officinalis* L.) is very well-known garden plants, producing large numbers of yellow-orange flowers over a long period. This species apart from its use as an ornamental, has traditional culinary and herbal uses. The aim of this paper was to find the best adapted variety and optimal row distance, concerning flowers and petals yield and quality. Trial was conducted in 2006-2007 at experimental field of Institute of Field and Vegetable Crops at Rimski Šančevi (φ 45°20' N, λ 19°51' E), Novi Sad, Serbia. The experimental design was a randomized complete block design with a split-plot arrangement, where the main plots were cultivars and the sub-plot row distances. There were 4 cultivars: 'Bački Petrovac', 'Orange King' (originated from Serbia), 'Plamen' and 'Plamen Plus' (from Czech Republic) and 4 row distances: 40, 50, 60 and 70 cm with constant 10 cm between plants in row. According to ANOVA year had significant effect only on dry flowers yield. Variety had very significant effect on fresh and dry petals yield, the Plamen Plus had the highest and Bački Petrovac the lowest. Row distance had very significant effect on fresh and dry flowers and petal yields, the values regularly increased from 70 to 40 cm, where was the highest. Interaction was very significant only between year and variety for all traits, in 2006 the best was Orange King and in 2007 Plamen Plus. The quality of dried flowers and petals was significantly affected only by varieties. Content of essential oils in flowers was in range from 0.32-0.50 ml/100, in petals 0.11-0.33 ml/100g and was the highest at Czech variety Plamen. Highest content of total flavonoids (11.92%) and total phenol substances (1.37%) in petals had domestic variety Bački Petrovac, while it's content in flowers was the highest in variety Plamen Plus.

Sm08.008

Effect of Picking Time on Essential Oil Yield of Ylang-Ylang (*Cananga odorata*)

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Ylang-ylang (*Cananga odorata*) (Lam.) Hook.f. & Thomson, a member of the *Annonaceae* Family is a tropical medium-tall tree reaching a height of 10-20 metres. It produces flowers nearly all year round and the peak season is in rainy season during May-September. Ylang-ylang flowers have a fresh, sweet and oriental floral aroma. The essential oil obtained from ylang-ylang flowers has long been used in fragrance and food flavoring industry and today it is a popular component of many legendary perfumes and aroma formulae in spa business for health and beauty. The study was aimed at finding the optimal harvesting time for ylang-ylang flowers to maximize the essential oil yield. There were 4 experiments: different time of picking and distilling; different stages and sizes of flowers. The results have shown that picking flowers at 8:00 am, 12:00 noon and 4:00 pm gave 0.42, 0.40 and 0.35% v/w while picking flowers at 8:00 am and the hydro-distilling process done at 9:00 am, 1:00 pm and 5:00 pm gave 0.45, 0.44, and 0.44 % v/w. Yet when the distilling time done at 9:00 am of the following day, it resulted in high amount of essential oil with 0.67% v/w. It was clearly seen that the stages and sizes of flowers affected the essential oil yield. The percentage of essential oil yield from the immature green stage, the mature greenish-yellow stage and the late yellow stage were 0.25, 0.41 and 0.31 % v/w. The large flowers (1.37 x 7.14 cm; 1.91 g/flower) gave the greatest amount of essential oil with 0.74 % v/w whereas the medium (1.20x5.87 cm; 1.56 g/flower) gave 0.47 % v/w. The flowers of small size (1.18 x 5.08 cm, 1.10 g/flower) gave only 0.16 % v/w. The main constituents of the extra grade of ylang-ylang essential oil extracted from hydro-distillation method analyzed by Gas chromatography/Mass spectrometry were as follows: geranyl acetate (18.28 %, benzyl benzoate (14.42%) germacrene D (10.92 %), trans-caryophyllene (10.71 %) and geraniol (8.44 %).

Sm08.009

Agronomic Production and Essential Oil of *Ocimum basilicum* L. in Different Systems, Fertilization and Season Cultivation

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Evaluations the agronomic production and yield of essential oil of *Ocimum basilicum* L. produced in different systems (field, greenhouse), different types of fertilizer (mineral and organic) and different seasons the year (spring-summer / autumn-winter). The experiment was conducted at Farm Experiment of the Gloria (FUU), Uberlândia, Minas Gerais state. The delineation was entirely randomized, in factorial design 2 x 2 with five repetitions. As source of nutrients have used chicken manure for the organic and NPK (4-30-16) for the mineral fertilizer. It was used the cultivar Maria Bonita and the spacing used was 60 cm x 30 cm. The beds had 10 plots each plot with 20 plants. Five plots received organic manure, and the other five, mineral fertilizer. The crops were taken in the morning on 19/11/2008 and on 20/05/2009, when the plants were in full bloom. The variables analyzed were height, length and width of leaves, fresh and dry mass, yield and composition of essential oil. The essential oils were extracted by hydrodistillation with Clevenger type apparatus, for two hours. The data were subjected to analysis of variance joint and means compared by Tukey test ($p \leq 0.05$). There was no interaction between cropping systems and two types of fertilizers for the two seasons the cultivation. Approximately 98% of the substances contained in the essential oil of basil were identified. The three most substances produced by cultivar were linalool, acetate linalool and 1,8 cineole. The type of fertilizer did not influence the production and yield of essential oil of basil, the two seasons the cultivation. Cultivation in a protected environment favored the growth of leaves and increased leaf area, and during the two seasons year, favored increased production of essential oil extracted from the biomass of the dry leaves of basil.

Sm08.010

Traditional Uses of Organic Medicinal Plant Species

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Medicinal herb species constitute a valuable resource for low-income residents. They can be found in Latin American front and backyards, tended by the household members in a co-operative manner, without usage of chemicals. In 1998, the Portuguese Tropical Research Institute has initiated field research in the Brazilian Amazon, focusing urban agriculture practices within the municipality of Belem. So far, five other cities have been investigated regarding traditional medicinal knowledge: Lima (Peru), Santiago (Chile), Mexico City (Mexico), Havana (Cuba) and San Jose (Costa Rica). Research results on the issue of organic medicinal herb production in urban and periurban areas will provide support and build confidence in the traditional uses of medicinal plant species. Hope is to contribute for a healthier urban environment and to present information for researchers and farmers engaged in the production, evaluation, or use of herbal medicines.

Sm08.011

Major Aroma Ingredients of *Lilium Oriental* 'Siberia' and its Effect on Human

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Flower aroma from *lilium* oriental hybrids is strong and easily identified. The volatile fragrance compounds released from fresh cut flowers of *lilium* oriental hybrids 'Siberia', were analyzed by dynamic headspace collection and gas chromatography - mass spectrometry (GC-MS). The main aromatic ingredients were Homomenthyl salicylate (25.98%), 1,6-octadien-3-ol,3,7-dimethyl Linalool (10.61%), 1-3-6-octatriene, 3-7-dimethyl-cis-ocimene (7.81%) and Hexadecanoic acid 1-methylethylester (6.54%). As related biological targets had been mensurated, lily aroma made subjects brain boost β wave amplitude and the rate of galvanic skin response (GSR) ascended. That shows that the subjects are caused sympathetic activity, which reacts the Linalool's effects. Linalool is a kind of monoterpene alcohols, has obvious stimulus effect on human biological and psychological targets. With the assistant of questionnaires, the study demonstrates that lily scent has a high recognition and most people describe the lily scent as relaxed, excited, enjoyable and other bonus words.

Sm08.012

The Relationships between Lavender Aroma Component and Aromachology Effect

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It is widely accepted that aromas from plant essential oils show some mental and physiological effects that are often called 'Aromachology' effects. However, there are few reports exhibiting the clear scientific evidence on aromachology effects of essential oil. In this study, we tested aromachology effects of essential oils from two lavender (*Lavandula angustifolia* and *L. intermedia*). Lavender essential oil is believed to have relaxing effects. POMS (Profile of Mood States) test was tried to reveal the mental effects of the oils on human volunteers. Some relaxing effects such as decrease of 'Fatigue' feeling were observed by giving aroma of both the lavender essential oils. Power spectral analysis on R-R intervals of heartbeat was used for evaluating the physiological effects of the lavender oils on human autonomic activity. Aroma of the lavender oil from *L. angustifolia* (called 'true lavender') caused an increase of human parasympathetic nerve activity, but the oil from *L. intermedia*

didn't show such an effect. To reveal key compounds causing such a difference in the physiological effect, volatile compounds of the essential oils were analyzed by GC and GC-MS. The similar contents of linalool and linalyl acetate were included in both the essential oils, but the content of camphor was higher in the oil of lavender. Since camphor is believed to have the ability increasing sympathetic nerve activity, this compound may offset the relaxing effects of linalool and linalyl acetate.

Sm08.013

Estimation of Genetic Parameters in a Linalool-Type Basil Population

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Basil (*Ocimum basilicum* L.) is an aromatic and medicinal plant species of great economical importance, mainly owing to its essential oil content. The available *Ocimum* ssp. genotypes vary considerably in the production and active principles of essential oil. This study aimed to estimate genotypic and phenotypic yield-related parameters and genetic gain after selection in three generations of selfing. The accessions were evaluated and selected based on individual plant selection in the S0 (original population) and S1 and S2 generations of selfing, for essential oil content and linalool content in essential oil. There were significant yield increases for both variables with gains of up to 234% for essential oil content and 71% for linalool content, indicating efficiency in the selection process. The estimates of the genetic variance and heritability, associated to high yield values, suggest the possibility of developing a new basil cultivar.

Sm08.014

Effect of the Different Plant Origin and Climatic Conditions on the Total Phenol Content and Total Antioxidant Capacity of Self-Heal (*Prunella vulgaris* L.)

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The cultivation methods for medicinal plants ought to guarantee higher yields of active constituents in the final products. However, due to the predicted climatic changes, the present agricultural systems have to be modified. According to previous reports, the quality and quantity of active constituents accumulated in medicinal plants are highly affected by climatic conditions. Climatic changes will result in several stress effects on the plants both under cultivation and in their natural habitats. In the last years it was proved that the accumulation of several terpenoids and phenol compounds can increase as a result of stress response reactions. Since phenol compounds can be regarded as "multifunctional" antioxidants due to stress effect, the amount of these chemical substances can raise significantly. Self-heal (*Prunella vulgaris* L.) populations were evaluated in different habitats of Hungary and Italy in 2007. According to our results, the total phenol content (TPC) as well as the total antioxidant capacity (TAC) of the samples was affected by the sunnier, warmer weather conditions in Italy. In fact, plants coming from Monte Pisani and from the Botanical Garden of Lucca were characterised by significantly higher TPC (0.6±0.05 and 0.7±0.01 mg GAE/ml, respectively) and TAC (0.91±0.19 and 1.14±0.06 mg AAE/ml, respectively) values than the Hungarian originated samples (average values of 0.41 mg GAE/ml and 0.59 mg AAE/ml of seven Hungarian locations). The altered climatic conditions (direct sun instead of half shadow, higher temperature) during the cultivation of the same genotypes resulted in a similar tendency. Comparing to the woodland natural habitats, in the cultivated plants the level of TPC (average values in the natural habitats: 0.30 mg GAE/ml and in the cultivated populations: 0.53 mg GAE/ml) and TAC (average values in the natural habitats: 0.52 mg AAE/ml and in the cultivated populations: 0.72 mg AAE/ml) increased significantly.

Sm08.015

Chemical Intraspecific Variability and Chemotypes Determination of *Rosmarinus officinalis* L. in the Region of Murcia

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Rosmarinus officinalis L. essential oil quantitative chemical composition depends, as it has been reported in several publications, on the geographical origin of this species. Major components previously identified in the essential oil, which define the chemotype of these plants are camphor, 1,8-cineol and α -pinene. However, the intraspecific variability detected among plants belonging to a reduced geographical area, as it occurs in the Region of Murcia (11,313 Km²) implies the necessity of defining the existence of different chemotypes and their relation to the climatic conditions in the growing area. On these bases, 31 wild rosemary populations (making a total of 152 individual plants) were prospected and analyzed. The chromatographic analysis allowed for the detection of 3 major different chemotypes, including: 1,8-cineol- α -pinene-camphor (representing a 18.42% of the total plants analyzed) followed by camphor-1,8-cineol- α -pinene (17.76%) and 1,8-cineol-camphor- α -pinene (13.82%). Minor chemotypes characterized correspond to: α -pinene-1,8-cineol-camphor (4.61%); α -pinene-camphor-1,8-cineol (3.95%) and camphor- α -pinene-1,8-cineol (3.95%). It is interesting to remark the existence of some plants with an essential oil rich in components as verbenone (13% of the oil), borneol (11%) and camphene (10%). For these shrubs, the essential oil chemotype is defined by the major components above mentioned plus these minor components. At the same time, the effect was determined by the altitude of the growing area on the essential oil chemotype definition. Regarding this, in areas located from sea level to 1000 m above sea level, the major component identified in the oil was 1,8 cineol (representing 49% of the plants analyzed) followed by camphor (27%). However, this situation changes in areas situated between 1000 and 1500 m a.s.l.; since the percentage of shrubs with camphor-1,8-cineol, and 1,8-cineol-camphor chemotypes were similar (40% and 38.5% respectively).

Sm08.016

Antioxidant Properties and Total Phenolics of Anatomical Parts of *Hypericum foliosum* Aiton

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Methanolic extracts from anatomical parts of the Azorean endemic *Hypericum foliosum* Aiton (aerial parts-AP; flowers-FL; old leaves-OL; young leaves-YL; stems-ST; stem bark-SB; root-RO and seed capsules-SC) were studied to evaluate their potential antioxidant activity and the basic classes of its bioactive compounds using commonly accepted methodologies. The results reveal that when the antioxidant activity determination is carried out by a single method the antioxidant potential could be underestimated, since significant differences both in phenolic content (TPC) and antioxidant activities were observed. Among all samples assayed, the ST showed the best overall results including a TPC of 386.88 mg of gallic acid equivalents/g dry extract which is twice the TPC of the majority of the other samples. On the other hand, the SC showed the lowest overall activity. The AP, SB and RO have higher amounts of phenolics and superior antioxidant properties than OL, YL and FL extracts in the majority of the assays. No encouraging results were obtained regarding to the inhibition of the oxidative damage of proteins with six extracts presenting a pro-oxidant role. A high correlation was found between TPC and antioxidant capacity, indicating an important role of polyphenols. The TPC showed a significant correlation ($P < 0.05$) with radical scavenging activity ($r = 0.756$), prevention of lipid oxidation ($r = 0.618$), superoxide anion activity (r

= 0.810) and the reducing power activity ($r = 0.828$). No correlation was found between TPC and both protein oxidative damage and iron-chelating ability which could be related to other substances such as polysaccharides, proteins or peptides in the extracts. These results reveal a promising plant, particularly the stems and stem bark, containing powerful antioxidants with different modes of action, for a more detailed future investigation of its potential pharmaceutical applications.

Sm08.017

A Comparative Study of Aroma Components of Wild Chrysanthemums in China

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Most *Compositae* plants have aromatic odor but different kinds of wild chrysanthemum have significant difference among aroma components. A dynamic headspace protocol was used to collect fresh floral scents of six wild chrysanthemums. By using the Thermal desorption Cold Trap Gas Chromatography/ Mass Spectrometry (TCT-GC/MS) technique, more than 30 components were identified from the volatiles, including the shared aroma components such as n-hexane, ethyl acetate, α -pinene and cineole. Different chrysanthemums have their own components too. Comparison of the volatile components in the six wild chrysanthemums showed that β -Phellandrene, nonanal and beta-elemene were special aroma components of *C. chaneitii* and 1,3,5-Cycloheptatriene, 2,6,6-trimethyl-bicyclo[3,1,1] hept-2-ene, trans-ocimene, cis-terpineol were characteristic components of *C. indicum*. Acetic acid, hexylester and D-Germacrene were own components of *C. indicum* var. aromaticum, while *C. vestitum* have six kinds aroma components, such as 1-Octene, Hexanal, hexenal, 3-Hexen-1-ol, 3-thujene and benzaldehyde. Benzene and 3,5-Heptadien-2-ol, 2,6-dimethyl were characteristic of *C. lavandulifolium*. And *Opisthopappus taihangensis* had two kinds characteristic aroma components, limonene and farnesene. It is shown that *C. vestitum* and *C. chaneitii*, *C. indicum* var. aromaticum and *C. lavandulifolium* were clustered into the same group on the dendrogram because of their similar aroma components, while *Opisthopappus taihangensis* was clustered into another group for its significant different aroma components from the other five kinds of chrysanthemums.

Sm08.018

Effect of Salinity Stress (NaCl) on Growth and Development and Chicoric Acid of *Echinacea purpurea* cv. Magnus

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Salinity is a growing problem in agricultural soils that affects plant growth, including medical plants, reducing their yield. On the land, the synthesis of active material in medical plants is clearly under the influence of environmental conditions and in stress conditions, the production of secondary material increases and consequently economical output rises. In this research, the effects of concentrations of 0, 20, 40, 60 and 80 mM/L of NaCl on amount of chlorophyll, Fv/Fm, fresh mass accumulation of root and aerial organ, number of root, diameter and length of root, number and surface of leaf, dry mass accumulation of root and aerial organ, sodium, potassium, calcium and magnesium in root and aerial organ and chicoric acid of root of cultivar Magnus (*Echinacea purpurea*) were investigated. The results showed that by increasing the salinity, all growth factors reduced significantly. Salinity increased the amount of sodium in leaf and root and reduced the amount of potassium and magnesium. Salinity increased significantly the amount of calcium of root and reduced the calcium of leaf. By increasing salinity, chicoric acid of root increased significantly.

Sm08.019

The Composition of Herbage Essential Oils from Natural and Culture of Endemic *Salvia cyanescens* Boiss. et Bal. in Turkey

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This research was carried out at Organic Farming Program, Mudurnu S.A. Vocational Higher School, Abant Izzet Baysal University and the Department of Field Crops, Faculty of Agriculture, University of Ankara in 2009. The plantation was established with natural cuttings of *Salvia cyanescens* Boiss. et Bal.; endemic to Turkey, in 2009 year. This year, only one cutting was done and the herbage samples for essential oil compounds were obtained from this cutting. Essential oils obtained by hydrodistillation from natural and culture of herbage were analyzed by GC-MS, Hewlett Packard 6890 N model, for determination of essential oil compounds. Essential oil ratio from natural and culture area was recorded as 0.06% and 0.05%, respectively. 17 compounds both in the natural herbage and the culture herbage representing 95.77% and 96.40% of essential oils, respectively, were identified. While β -pinene was the compound with the highest value (17.55%) in the natural area essential oil, β -cubebene was determined as the highest value (24.94%) component of the culture area essential oil.

Sm08.020

Chemical Characterization of *Rosmarinus officinalis* Wild Populations Essential Oil from Alcarria Region (Spain)

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The quality and commercial value of an aromatic or medicinal plant is fairly related with its chemical composition. Secondary metabolites are compounds which are not directly involved with the natural plant growth. Their production is known to be influenced by many factors, both environmental and genetic as a response to particularly stressful situations (high temperature, low water availability, herbivores pressure) or as an information exchange mechanism. Our work is focused on the chemical characterization of rosemary essential oil from 30 Spanish wild populations. This bio-prospection has been carried out in Alcarria region, placed in the Centre of the Iberian Peninsula, where plant material was collected at full-flowering time. In this area, rosemary is a native species which appears as an attractive crop due to its adaptation to the local climatic and soil conditions. Essential oil was extracted from rosemary leaves and flowers by hydrodistillation and was analyzed by gas chromatography. The first results show an average essential oil yield of 2.05% (± 0.25). Sixty-two different compounds have been identified, accounting for 96.25-100% of the total oils. Terpene hydrocarbons α -pinene (8.01-16.75%), camphene (5.00-8.73%) and β -pinene (6.02-13.09%) and oxygen terpene derivatives 1,8-cineole (13.28-32.36%) and camphor (13.74-34.61%) are the most representative ones. The aim of this research is to obtain as much information as possible about natural and biochemical available resources to start with breeding programmes of wild growing populations in order to provide an appropriate plant material necessary for an adequate cultivation technology.

Sm08.021

Phenolic Fraction Composition
(Phenolic Acids and Flavonoids)
of *Salvia lavandulifolia* Wild Populations
from Alcarria Region (Spain)

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Spanish sage (*Salvia lavandulifolia* Vahl.) is a perennial undershrub native from Iberian Peninsula. It mainly grows in dry areas where there is a limestone formation with little deep soil. These low water and soil requirements would make them an attractive species for local farmers as an alternative to traditional crops in marginal lands. It needs an appropriate plant material to achieve homogeneous productions. Our study involved the collection, chemical characterization and conservation of 30 Spanish sage wild populations from Alcarria region (Spain), in order to get data about their chemical composition, which will be used in future breeding and selection programmes. All the samples have been collected in full-bloom time. They have been hydro distilled to extract their essential oil. After that, the methanolic extract of the distillation residues has been analyzed with HPLC method to determine their phenolic fraction composition. The antioxidant capacity of phenolic acids and flavonoids is well known. This biological activity has a preventive effect against cardiovascular diseases and cancer, making the plants with high contents on them very interesting for phytopharmacy, cosmetic and food industry. First HPLC analysis results show an average flavonoids concentration on Spanish sage distillation residues of 120.87 mg/Kg (57.69-228.62 mg/kg) being Kanferol (29.95-158.50 mg/kg) and Quercitrin (12.59-75.37 mg/kg) the majority ones. Regarding phenolic acids, the average concentration of all the samples is 11.66 mg/Kg, going from 6.11 to 25.76 mg/kg. Rosmarinic acid is the main one (5.75-20.81 mg/kg). Finally, Carnosic acid and Carnosol have been measured separately as these are described on bibliography as the most representative phenolic compounds in Spanish sage, in our study, they have been found in an average concentration of 6.14 mg/kg (2.75-14.40 mg/kg).

Sm08.022

Temulawak (*Curcuma xanthorrhiza* Roxb.):
Standardization Activity and Chemical Profile

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The research is aimed to develop the standardized *Curcuma xanthorrhiza* Roxb. as raw material for the herbal medicine. The research consists of two steps. The first step is standardization of *C. xanthorrhiza* material production through variety selection and modification of growth environment. The second step is the separating of temulawak bioactive compound from the matrix through separation methods selection. The parameters of these activities are active compound content (curcuminoid and xanthorrhizol), Brine Shrimp Lethality Test (BSLT), and anti oxidant test. The result showed that the promising line of *C. xanthorrhiza* A is the best promising line of temulawak because it has high xanthorrhizol and curcuminoid productivities. Moreover, Cileungsi is the most appropriate growth environment for *C. xanthorrhiza* cultivation with high bioactive productivity. The inorganic cultivation technique produced an enhanced xanthorrhizol and curcuminoid productivity. Masseration with ethanol 96% is the best extraction method in separating bioactive from the *C. xanthorrhiza* matrix. The chemical profile and the activity of the extract also will be explored in this paper.

Sm08.023

Networking on Conservation and Use of
Medicinal, Aromatic and Culinary Plants Genetic
Resources in Portugal

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Genetic Resources are crucial to support humankind wellbeing by contributing to increase the income of the rural populations and, thus, their general welfare, by maintaining the traditional sustainable agricultural systems. However, their importance goes far beyond the immediate economical value, as it has also drawn considerable interest at scientific and political levels as important elements contributing to local and global food security and quality. Being a repository of unforeseen potentialities, MAP GR should be studied and preserved for the benefit of present and future generations. In the past decades, research programmes on MAP GR have basically been focusing on biochemical evaluation of wild material. Nonetheless, in the last decade, efforts have been done to preserve this genetic material *in situ* and *ex situ*. Since the year 2000, several researchers have pulled synergies and joined efforts and have proposed collaborative networks on MAP GR. It was possible to define a first MAP Programme for the *in situ* and *ex situ* conservation, ethnobotany, characterization, evaluation in support of the promotion of their sustainable utilisation. In Portugal, the MAP collection preserved in *ex situ* conditions (field and seed collections) and *in vitro*, adds to a total of 1,224 accessions belonging, inter alia, to the families *Liliaceae* (3%), *Apiaceae* (2%) and *Lamiaceae* (0.7%). So far, twenty-seven species belonging to these families were characterized, evaluated and multiplied, and a total of 1,500 ethnobotanical questionnaires were elaborated at national level based on 1,000 interviews. Through these established networks it was possible to increase the amount and effectiveness of the knowledge about this important pool of genetic material and engage in systematic morphological characterisation and biochemical evaluation. The acquired integrated and complementary knowledge will allow for and the support of MAP future actions, associating the understanding and management of biodiversity and genetic resources conservation and utilisation.

Sm08.024

Friends or Foes? Ornamentals - Medicinal
Plants - Poisonous Plants

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Although some people are not aware of it, we can constantly come across medicinal plants and poisonous plants alike, often in the form of ornamentals - inside or in the surroundings of our homes, schools and working places. Often it is difficult to clearly distinguish between medicinal / poisonous / ornamental plants. In case of several species, such as lavenders, oreganos and sages, a large number of taxa are available for the cottage garden both as ornamentals and as sources of home remedies. Our investigation directed at the medicinal value of ornamental sage cultivars revealed that their volatile compounds were the same as those of common sage, only the ratio of components differed. The physiological effect of several plant derivatives is dose-dependent: small doses can be used for therapeutic purposes, while larger doses exert a toxic effect on humans and/or animals. E.g. the extracted cardiac glycosides of widely planted

Convallaria, *Digitalis* and *Nerium* species are used in treatment of cardiac failure, but the plants are poisonous if consumed or used as tea drugs. The tropane alkaloids of *Datura* species, often planted for the decorative value of the flowers, are applied in medicine, but consumption of the plant, either intentionally or accidentally, may lead to intoxication. In our experience not only the various organs of *Datura* sp.; but also the floral nectar contains the alkaloids. Attractive as they are, some of our most popular indoor ornamentals, e.g. *Anthurium*, *Dieffenbachia*, *Philodendron* and *Spathiphyllum* species are poisonous, being especially dangerous for children, who are inclined to taste various plant parts. According to our survey conducted in Hungarian nurseries and kindergartens, the little ones are exposed to the danger of intoxication by touching or consuming poisonous plants like *Laburnum anagyroides*, *Taxus baccata* and *Thuja* spp.; often planted as ornamentals in the playing grounds.

Sm08.025

Experiences Improvement the Herbal Crops Farming Practices and Empowerment the Farmer's Institution in Some Production Centers of Indonesia

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Some location in Indonesia have the good condition and potential enough for herbal crops farming because of the suitable and favorable agro-ecosystem, availability of land and water resources, and low competition with other crops. Many local social culture of the people and also the community life style have been used the herbal crops as material for medicinal, refrigerant, beverage and cosmetic purposes since long time ago. Moreover, jamu was declared as Indonesia heritage for traditional herbal which have been popularly consumed by the people for alternative and traditional medicinal, cosmetic, body refresher, etc. These conditions have supported to develop the herbal crops farming in Indonesia. Mostly the herbal crops which have been developed in some production centers in Indonesia is the rhizomes, such as; ginger, galanga, Indian galangal, turmeric, aromatic wild ginger, java turmeric, black turmeric, and Chinese key. As raw material for medicinal and jamu industries, the quality and characteristic of the product should be maintained properly in order to be conformed to the need and standard of the industry. Supporting to this matter, some actions have been carried out for farming practice improvement are; 1) formulation the GAP/SOP, 2) utilization good variety and selected seed based on the requirement of the industry, 3) selection of suitable location by considering the technical and environmental aspects (herbal crops belt development/Kawasan Tanaman Obat), 4) application of the integrated farming system approach, 4) application and promotion of organic farming and sustainable development, 5) improvement and practicing good post harvest handling, 6) primer processing on the selected product. By conducting that efforts and actions have been resulted in increasing the herbal crops production to meet the requirement of the industries, as well as improving the capabilities and capacities of the farmer's institution to produce the good quality product.

Sm08.027

In vitro Flowering in Cultures of *Daucus carota* subsp. *Halophilus*, a Portuguese Endemic Carrot

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Daucus carota subsp. *halophilus* is a carrot wild-relative growing in some regions of Portugal as an endemic species. As other members of the *Apiaceae* family, it produces essential oils that have been characterized in a previous work. Antifungal and cytotoxicity tests showed that the oil with the highest amounts of elemicin displayed the strongest antifungal activity without side effects on mouse skin dendritic cells. Protocols for the *in vitro* propagation of this species were also established by our group through axillary shoot proliferation and somatic embryogenesis. In this work

the conditions for *in vitro* flowering are described. Shoots were established from axillary shoots of field-growing plants on a MS medium containing 1.5 mg/L of N6-benzyladenine and 0.5 mg/L of indol-3-acetic acid and proliferating in the same culture medium start to develop inflorescences after 6 months of culture under a 16h light/8h dark photoperiod (25 °C). Repeated subcultures using the basal parts of the proliferating shoots, in the same culture medium, results in new cycles of shoot and flower formation each four weeks. These cultures have been maintained for 18 months without loss of the flowering potential. Apart from the dimensions, inflorescences produced *in vitro* closely resemble those formed in natural conditions. Ovules and anthers produced *in vitro* have also the same morphological characteristics that those occurring *in vivo*. Moreover, acetocarmine squashes of the anthers and fluorescence microscopy analysis using DAPI showed that the *in vivo* and *in vitro* anthers produced pollen grains quite similar. However, in some cases, supernumerary divisions of the pollen cells have been observed. Attempts to achieve seed formation and to complete the life cycle of *Daucus carota* subsp. *halophilus* in laboratory conditions are being pursued. This would be very useful for plant conservation, to improve conditions for the propagation of this taxon and for essential oil production.

Sm08.028

In vitro Cultures of *Pogostemon cablin*

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Pogostemon cablin or patchouli is a native to tropical regions of Asia. It is a perennial aromatic herb belongs to *Lamiaceae* family. The plant has been cultivated for its essential oil which has been used in perfumery and food industries. The herb can also be used to treat pharynx pain, asthma, cough and fever. This study was conducted in order to determine the effect of various hormones combination (NAA and BAP) on leaf, stem and lateral bud explants of *Pogostemon cablin* using tissue culture technique. Result shows there was a significant difference in microshoots development and callus formation using stem and lateral bud as the explants in various combinations of hormones. For the microshoots growth, the optimum hormone combination was 0.5 - 1.0 mg/L NAA with 0.5 - 1.0 mg/L BAP. On the other hand, for callus (unorganized and undifferentiated tissues) formation, the optimum hormone combination was 2.0 mg/L NAA and 0.1 - 1.0 mg/L BAP. A friable and whitish callus was obtained from MS (Murashige and Skoog) medium supplemented with higher auxin (NAA) concentration. By comparison, sucrose concentration at 40 g/L in MS basal medium gave the optimum microshoots development whereas sucrose concentration at 20 g/L gave the optimum callus formation. The *Pogostemon cablin* microshoots and callus obtained from this experiment can be used to analyze the phytochemicals and biological activities.

Sm08.029

A New Protocol for Propagation and Development of the Greek Endemic Species *Origanum dictamnus* L.

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Asexual propagation and cultivation in hydroponical system and in a pilot field were studied, in the frame of a research project aiming at the sustainable utilization of the Greek endemic species *Origanum dictamnus* L. Rooting of softwood cuttings was tested using two different substrates and three concentrations of IBA (0, 1000, 2000, 4000 ppm). The rooting percentage, the number of roots and the root length were measured. Best results (90-95% rooting) were observed in the substrate perlite: peat (3:1) at 1000 ppm IBA. Developed plants were planted in a pilot field where four different organic fertilizations treatments were applied. Height, diameter, fresh and dry flower weight per plant and per hectare were measured. The highest dry flower production was achieved by the addition of Agrobiosol, an organic fertilizer consisting of organic matter (80-90%), total N (6-8%), P₂O₅ (0.5-1.5%) and K₂O (1-3%) (first year's production was 1170/he while the second was

increased at 2238 kg/he). Plants were also cultivated using hydroponical systems. The effect of three different substrates (perlite, peat:perlite, zeolite:perlite) and two irrigation levels on the growth of the plants were studied. Height, diameter, fresh and dry flower weight were measured. Best results were noticed at the peat:perlite substrate (first year 77g dry flower weight/plant and second year 121.86 g).

Sm08.030

Callus Induction of Kind of Parasitic Plants of Herbal Medicine

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Cynomorium songaricum Rupr, a kind of parasitic plants parasitize to the root of Nitarias, is an extensively used herbal medicine. The study proposed to induce *callus* from the seed of *C. songaricum*. The seed of *C. songaricum*, collected from Ordos (longitude 108.7°, latitude 39.83°), were treated with thermal shock processing temperatures of 30 to 70 °C using incubation time from 20 to 100 min. Then surface-sterilized seeds removed testa were used as explants to initiate *callus* on B5 medium supplemented with different contents of 2,4-dichlorophenoxyacetic acid (2,4-D), kinetin(KT) and Gibberellic Acid (GA3). *Callus* appeared after 25 days culturing in dark at 23 °C. *Callus* was fast-growing and pure white, which volumes were as three times big as seeds in the first five days. But *callus* turned to brown and grew slowly in the next five days, eventually to death about 25 days. Experiment set up to repeat three times, each time repeating handle up to 100 seeds. The induction frequency was about 10% to 20%. The seed of *C. songaricum* suffers deep dormancy for several reasons. Firstly, it is nut of hard seed coat preventing absorption of water and breath of air. Secondly, it has a component of high contents inhibiting germination as ABA. Last but not the least, embryo of the seed is immature which could process a long time of after-mature to germinate. Thermal shock may accelerate metabolism to break dormancy of the seed by inducing hormone-relate enzyme. Furthermore the use of GA3 helps to overcome the dependence of seed to host. This study established a method of seed *callus* induction for the first time, from which may form haustorium of *C. songaricum*. It may do some help to investigate parasitism of *C. songaricum*.

Sm08.031

Temulawak, Challenge and Development to Phytopharmaca Status in Indonesia

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The cooperation between A+B+C+G (Academicals, Business, Government, and Community) to develop herbal products of Indonesian origin has been developed recently. The aim is to have the quality, efficacy and safety issue for Indonesia origin products which is controlled from seed to clinical trial. The collaboration of cooperation including in Agricultural, Phytochemistry-Pharmacology, and Pre Clinical - Clinical Trial. The team should be committed and focused in their role of working area. SOHO Group, one of the best local pharma company in Indonesia, is committed to creating and developing herbals of Indonesian origin. Currently we are focusing on Temulawak or Curcuma xanthorrhiza development not only for the Indonesia market but also for developed countries. With the support from the team from local government, including Agricultural Ministry and Balitro (Indonesian Medicinal and Aromatic Crops Institute), from Academicals (Bogor Agricultural University), and Community such as the farmer thus will be good model of development to have such a quality, efficacy and safety herbal product from Temulawak. It was known that Indonesia as a tropical country enriched with its mega biodiversity, have a great number of plant and some of them known as medicinal herbs. Temulawak, one of the ancient Indonesia herbal origin has been used by ancient Indonesian people as their herbs remedies to keep their health, prevent and recovery from disease. It is become a challenge for Soho to develop more for Temulawak and have standardized quality from 'seed to patient'. Therefore Soho is building some good model of development involving the A+B+C+G concept. The aim is not only to achieve good quality, efficacy and safety products but also to improve the agro-economy within the farmers around the Temulawak plantation.

Soho aim is not only for local Indonesia market but also for international market. In order to bridging Indonesia product within international market, Soho is willing to put huge effort in the development of both in the agricultural seed development and also in research & development thus including clinical trial. Soho is also having partnership with institution in developed countries on research and analysis. Beside Soho already got some cooperation with some company in developed countries to market for the product. After the indication and photochemistry already settled, we can move forward to clinical trial process and got marketing approval within the indication. In the future, Soho is willing to build herbal centre and research development of Temulawak. This aim is to have a good role model of herbal development in Indonesia. This project will involve all team from government, academicals and community. Hopefully, Temulawak can be Indonesia ginseng in worldwide and well known internationally as good quality, efficacy, and safety herbal product.

Sm08.200

Assessment of *Achillea cartilaginea* Introduced from Wild to Field Collection

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The raw material of *Achillea millefolium* L. is one of the oldest and most important drugs in pharmacopoeias of many countries. There is little known about other yarrow species which could be used as new sources of herb material in pharmacy. The aim of the research was to assess phytochemical diversity of *A. cartilaginea* Ledeb. ex Rchb. introduced into field collection and to select valuable accessions for further cultivation. The essential oils from flowers and leaves were isolated by hydro distillation and then were analysed with Fisons 8261 gas chromatograph with flame ionisation detector (FID) on a fused silica capillary column, (25 m × 0.2 mm × 0.5 µm). Qualitative analysis was based on the comparison of retention indices and mass spectra. The fraction of oxygenated monoterpenes was dominant in essential oils. The flower oils according to the dominant compounds were attributed to camphor, 1,8-cineole and β-thujone+ chrysanthenone chemotypes. The leaf essential oils demonstrated great variation in dominating compounds. The study provides information about the composition of phenolics in ethanolic extracts of *A. cartilaginea*. Phenolic compounds were performed using a liquid chromatographic Waters 2690 Alliance HPLC system equipped with a polymeric 5µm Ascentis™ RP-Amide analytical column (150 × 4.6 mm). Compounds were identified comparing eluting retention times and UV spectra with those of authentic standards. The total content of identified compounds varied from 9.56 to 15.03 mg/g in flowers, from 24.49 to 64.05 mg/g in leaves and from 4.67 to 13.10 mg/g in stems. Chlorogenic acid and five flavonoids, namely luteolin-7-O-glucoside, rutin, apigenin-7-O-glucoside, luteolin and apigenin, were identified in plant material. The flowers accumulated higher amounts of luteolin-7-O-glucoside, apigenin-7-O-glucoside, luteolin and apigenin while leaves were found to be superior with regard to chlorogenic acid and rutin. *A. cartilaginea* accessions have potential value as a primary source for further selection.

Sm08.201

Fennel Botanical Characteristics, Chemical Composition and Antibacterial Potential of Essential Oils Extracted from Seeds of Two Varieties of Tunisian Fennel (*Foeniculum vulgare*)

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Besides his first feature food, fennel (*Foeniculum vulgare*) has been known since antiquity for its many healing properties and its relative wealth in essential oil, two features that make this plant an interesting case study. While the second is generally preferred in rural areas. Based on the results of a preliminary literature review, we found that fennel used for therapeutic purposes in Tunisia comes in two varieties:

wild and cultivated, the former being used mainly in urban areas; while the second is generally preferred in rural areas. We are interested in the first part of this work the extraction and chemical characterization of essential oil of fennel seeds of two varieties considered. For this, we used the technique of hydrodistillation and then analyzed by gas chromatography. Almost all of these compounds (99.7%) were identified through the intersection of three different criteria (retention index on nonpolar phase, retention index on polar phase and mass spectrum). The main results have highlighted the overall presence of the same compounds in the samples analyzed, albeit at different levels depending on the variety studied, enabling us to assert that the two varieties studied belong to the subspecies *Cappillaceum*, Mays that they differ in their chemotypes (chemotype trans-anethole “for the variety grown, and” chemotype estragole “for the spontaneous variety). The second part of this work has been devoted to the study of the antibacterial activity of essential oil extracts obtained. Two different methods were used for the determination of this activity: method in liquid medium and method on solid medium. The results obtained show that the two varieties studied have antibacterial effects against *Escherichia coli* strains and *Enterococcus faecalis*, the difference in doses could cause this effect. Thus the wild variety is more interesting since it has a stronger effect than the cultivated variety.

Sm08.202

The Influence of Chitin on Growth and Chlorophyll Content of Two Herb Plants, *Anethum graveolens* L. and *Eruca sativa* L.

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The effect of chitin soil amendment was studied in the characteristics of organic glasshouse cultivation of *Anethum graveolens* L. and *Eruca sativa* L. plants. The seeds were sown in potting soil, covered with vermiculite and remained in the growth chamber until the first real leaves appeared. The seedlings were transplanted in pots filled with the following substrates: peat, peat and chitin (2g/l), peat-sand (2:1v/v), peat-sand and chitin (2:1v/v+2 g/l chitin). Chitin in the peat substrate did not affect the length and weight of the leaves and shoot, as well as of the entire lemon balm plant, while at the peat-sand substrate it increased the corresponding sizes. It also did not affect the weight and height of the rocket plant, while it increased the fresh and dry weight of the shoots. In the peat-sand substrate chitin increased the fresh and dry weight of the leaves, shoots, root and of the whole plant, as well as the length of the leaves and the plant height. The peat and chitin substrate increased the total chlorophyll content in the rocket plant's leaves by 73%, and in the peat and sand substrate by 39%, in comparison to the corresponding non-chitin substrates. This total chlorophyll increase in the presence of chitin in both substrates is due to both a and b chlorophylls. In the peat substrate chitin increased the fresh and dry weight of the leaves, shoots and of the whole dill plant, while in the peat and sand substrate no important change is remarked. The peat and chitin substrate increased the whole chlorophyll content in the dill plant's leaves by 13%, and in the peat and sand substrate by 15%, in comparison to the corresponding non-chitin substrates. This total chlorophyll increase, in the presence of chitin in both substrates is mostly due to chlorophyll a.

Sm08.203

Investigation of Volatiles from Saffron Flower and the Antioxidant Effectiveness

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Saffron (*Crocus sativus*) has a flower with three characteristic reddish colored stigmas and yellow stamens, and brilliantly hued in lilac or mauve. The dried stigmas are used as an additive for foods, beverages, and as a coloring agent in cooking, as

well as for medicine to treat a wide range of ailments, including stomach upsets, bubonic plague, and smallpox. The aroma of the stigma is distinguished by a hay-like or medicinal odour, which is from the constituent safranal, while stigma extracts exhibit high antioxidant and free radical scavenging activities. A number of reports have been presented regarding the constituents and effects of the Saffron stigma, whereas investigations of the whole Saffron flower are limited. In the present study, we investigated the aroma and effects of the Saffron flower as a whole. In autumn, purple buds appear and the Saffron flower develops a brilliant pastel shade of blue with a touch of purple-red, along with an enchanting scent that resembles that of roses, without a medicinal aspect. Using head-space techniques as well as solvent extraction analyses of the scent of Saffron flowers, we identified 2-phenylethyl alcohol as the main constituent, along with other newly identified components that conjure up the image of the scent of roses. In addition, extracts from the flower revealed highly antioxidant activities. To assess the antioxidant properties of aroma extracts from Saffron flowers, we evaluated the constituents using a DPPH method and the results showed some active components, including safranal. Furthermore, we used a contingent negative variation method to determine the psycho-physiological effects, which indicated that the Saffron scent has a relaxing effect.

Sm08.204

Quantitative Effects of Temperature and Light Intensity on Accumulation of Bioactive Compounds in St. John's Wort

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The quantitative effects of temperature and light intensity on accumulation of naphthodianthrone, phloroglucinol derivatives and phenolics were examined on greenhouse-grown St. John's Wort (*Hypericum perforatum* L.). Plants were grown in greenhouse separated into two parts: shaded by 50% transparent polyethylene cover and un-shaded. Temperature and light intensity were measured daily using a Sato Keiryoki MFG R-704 thermo hydrograph and a Delta-T Sun Scan Canopy light analyzer. During experiment plants were harvested weekly and assayed for the chemical compounds concentrations by HPLC method. According to the results, increase in temperature from 24 °C to 32 °C and light intensity from 803.4 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ to 1618.6 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ determinate continuous increase in contents of bioactive compounds. Multi regression analyses were performed to describe the quantitative effects of temperature and light intensity on accumulation of analyzed compounds. The relationship between temperature & light intensity and accumulation of phytochemicals was exposed by following regression equation: $\text{SMC} = [a + (b_1 \times t) + (b_2 \times l) + (b_3 \times l^2) + (b_4 \times (t \times l))]$, where SMC – secondary metabolite content, t - temperature (°C), l - light intensity ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), and a, b₁, b₂, b₃ and b₄ - coefficients of the produced equations. The simple equations were developed for predicting the contents of hyperforine, hypericine, pseudohypericine, amentoflavone, apigenin-7-glucoside, hyperoside, kempferol, rutin, quercetin, quercitrin, and chlorogenic acid in plant material of St. John's Wort. Our results suggested that temperature and light are important environmental factors to optimize the raw material production of St. John's Wort. The mathematical models produced in the present study could be applied as useful tools for prediction of content of phytochemicals and standardization of plant materials.

Sm08.205

Chemical Variability of Wild *Rosmarinus officinalis* L. from Algeria

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Rosemary (*R. officinalis* L.) is a Mediterranean shrub belonging to the *Lamiaceae* family. Wild rosemary, due to its bioactive properties, has been cultivated by local farmers, with no previous selection, for a long time. Specifically in Algeria, it occupies 100,000 ha of the territory; however in spite of that, there is an important lack

of knowledge about the chemical variability of this wild rosemary. On the bases of these statements, a total of 15 wild Algerian rosemary populations have been analyzed considering their essential oil yields (EO) and polyphenolic extract antioxidant activities (AA). Samples were harvested from natural populations located at 30 Km from the South-west of Saida. Statistically significant differences were detected among the EO yield of the different populations and as well as among their antioxidant activities. Essential oil yield (v/w) ranged from 1.36% to 2.16%. To describe the antioxidant properties the free radical scavenging activity using the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•) test, and the Ferric Reducing Antioxidant Power (FRAP), were assayed. From the two methods applied, only FRAP showed a linear correlation ($p < 0.001$; $r = 0.817$) with respect to the total phenolic content of the extracts. However, the test which highlights the biggest differences among the polyphenolic extracts was the DPPH• assay. These results will allow pre-selecting the best populations regarding the EO yield and the highest AA. Related to this matter, populations I (2.16% EO yield; 11.12 mg gallic acid/g dry extract; 9.84 mM Fe^{2+} /mg dry extract; 38.78 μg dry extract/mL MeOH); IV (1.64% EO yield; 27.63 mg gallic acid/g dry extract; 11.51 mM Fe^{2+} /mg dry extract; 61.52 μg dry extract/mL MeOH) and VIII (1.57% EO yield; 11.43 mg gallic acid/g dry extract; 9.98 mM Fe^{2+} /mg dry extract; 30.54 μg dry extract/mL MeOH) will undergo vegetative propagations for their subsequent establishment as a commercial crop.

Sm08.206

Chemical Characterization of Plant Genetic Resources of Gentian (*Gentiana lutea* L.) in the Counties of Laciana and Babia (Spain)

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Plan 42 is a project which aims to raise awareness in residents of Castile and Leon municipalities about alternatives to the use of fire and searches for viable models of economic development linked to the mountain. In this sense there has been an agreement between different institutions to study and regulate the use of *Gentiana lutea* L. and its marketing opportunities for Cabrillanes, Villablino, Palacios de Sil and Murias de Paredes municipalities, located in the northwest province of Leon in the southwestern boundary of the Cantabrian Mountains. Gentian is distributed throughout the central and southern Europe and Asia Minor mainly in mountainous regions of upper montane and subalpine pastures. In Spain it grows in grasslands, heath lands and clears over 800 m, mainly in Pyrenees and Cantabrian Mountains, being less common in central and southern systems. It has been traditionally harvested for its medicinal properties due to their bitter compounds such as gentiopicroin and amarogentin, located in greater concentration in the periderm of the root and rhizome, destined for the pharmaceutical and liquor industries. Samples were collected from 88 populations of both soil and plant material. Roots used for studies on chemical variability of the species and seeds for germination studies and germplasm conservation. Gentian is common in deep siliceous soils, turbo-sandy, sunny, compact and dry. The soil samples analyzed, are light-textured (loamy sand mostly), pH from acidic to very acidic (around 4) and high in organic matter content (always above 10%, and a high number of plots to 20%). The average quantities of amarogentin and gentiopicroside are 1.31 and 23.25 ppm. Respectively, with samples that exceed 30 ppm of gentiopicroside and 2 ppm of amarogentin. It is also remarkable its high content of flavonoids.

Sm08.207

Evaluation of Variability of Morphological Traits of the Selected Caraway (*Carum carvi* L.) Genotypes

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Caraway (*Carum carvi* L.) is one of the most important medicinal plants cultivated in Poland on area 8000 ha. The morphological traits, such as the number of branches and lateral shoots, the number of umbel per plant or seed size have a great influence on fruit yield. The aim of the study was to evaluate some morphological traits of the selected caraway genotypes. In the experiments done in 2008 and 2009, 25 selected caraway genotypes originated from: European botanical gardens (18), two cultivars: 'Rekord' and 'Konczewicki' and our own breeding strains were tested. The obtained results showed that the objects in caraway collection varied in terms of all tested morphological traits. The plant height ranged from 71.5 cm (Reykjavik) to 107.8 cm (cv. 'Konczewicki'). Number of branches on the main stem was from 5.3 (Reykjavik) to 10.0 (Jena). The number of lateral shoots ranged from 9.8 (Reykjavik) to 21.5 (strain 9/10). Leaf length was from 11.9 cm (Lozanna) to 29.1 cm (cv. 'Konczewicki'). The number of umbels per plant was from 91.4 (Reykjavik) to 251.9 (strain 9/10). The fruit yield ranged from 14.2 g (Reykjavik) to 48.5 g (cv. 'Konczewicki'). The weight of 1000 seeds was from 1.81 g (Salzburg) to 3.31 g (strain 9/1). The advanced breeding materials performed better characteristics of these morphological traits, which affect fruit yield. The caraway genotypes evaluation will make it possible to choose the appropriate genotypes for further breeding program.

Sm08.208

The Impact of Cultivation Techniques on Bioactive Compounds in the Aerial Parts of *Echinacea purpurea*

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Echinacea purpurea is an important medicinal plant and is used to treat infections, to aid in wound healing and to enhance the immune system. The active principles of *E. purpurea* include secondary metabolites such as alkamides and phenolic acids. Many secondary metabolites make up the defence system of plants and hence the contents of bioactive secondary metabolites in plants may change as a reaction to stress. As a result a part of this study was to investigate how nutrient deficiency affects the content of alkamides and phenolic acids in the aerial parts of *E. purpurea*. Other factors, which may affect the content of bioactive compounds is the time of harvesting the plant material. Therefore another part of this investigation was to determine how different harvest times around flowering affects the content of alkamides and phenolic acids in the plants. *Echinacea purpurea* was cultivated in 2008 and 2009. The 2008 plants were exposed to 0, 100 or 200 kg N/ha and aerial parts were harvested in August whereas the aerial parts of the 2009 plants were harvested at three different development stages during the growing season (July to late August). Alkamides and phenolic acids were extracted from fresh frozen aerial parts with methanol-water (90:10) and identified by liquid chromatography electrospray ionization ion-trap mass spectrometry (LC-ESI-IT-MS/MS) combined with photodiode array detection (PAD) and quantified in extracts by reverse phase HPLC-PAD. Major alkamides were identified as undeca-2E,4Z-diene-8,10-diynoic acid isobutylamide, dodeca-2E,4E,8Z,10Z-tetraenoic acid isobutylamide and dodeca-2E,4E,8Z,10E-tetraenoic acid isobutylamide whereas cichoric acid was the dominant phenolic acid. Preliminary analyses shows, that the contents of alkamides and phenolic acids increases due to higher concentration of nitrogen and that the different harvest stage affect their content of potential bioactive alkamides and phenolic acids. Conclusive results will be presented and discussed at the IHC.

Sm08.209

Chemical Variability of Great Burnet (*Sanguisorba officinalis* L.) Growing Wild in Poland

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Great burnet (*Sanguisorba officinalis* L.) is a perennial belonging to *Rosaceae* family. In Poland it usually occurs on the wet piedmont meadows. In the people medicine the herb and undergrounds organs of these plants were used as hemostatic in gastrointestinal disorders. Nowadays the extracts from the great burnet are applied in the treatment of hemorrhage and in diarrhoea. The main active compounds of the great burnet are tannins and phenolic acids. The aim of investigation was to compare ten populations of great burnet wild growing in different areas of Poland in respect of phenolic compounds accumulation. Quantitative and qualitative analysis was performed by HPLC. The investigated populations differed distinctly in the content of particular phenolic compounds. Differences between underground and aboveground organs also were found. Catechin, epigallocatechin, epicatechin, epigallocatechin gallate, gallic and ellagic acid were found in the roots whereas rosmarinic, caffeic, chlorogenic, elagic and gallic acids in aboveground organs.

Sm08.210

Poppy Cultivation in the Slovak Republic

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Poppy (*Papaver somniferum* L.) is a traditional crop in Slovak Republic and its cultivation has long-standing history. The recent conditions of poppy cultivation modify the Law 139 from 1998 about narcotic and psychotropic components and products. In order to this rule it is possible to cultivate of poppy on land more than 100 m² on a base of permission from the Slovak Ministry of Health. The large-scale cultivation of this special crop is concentrated in the West Slovakia, in nearness of the pharmaceutical company Zentiva, Co. in Hlohovec. Farmers prefer a combination method of poppy production: seeds for food purposes and dry capsules for pharmaceutical industry. Poppy production areas were from 386 to 2,714 hectares during last 10 years. Yield of seed are usually from 0.28 to 0.73 ton per hectare. Good agricultural practice and own Slovakian poppy varieties are very suitable background for a high yield potential of seeds (about 2 tons per hectare). Poppy capsules are as a secondary product, which is very important raw-material to our pharmaceutical industry. Their yields fluctuate according to the season and customer requests from 300 to 500 kg per hectare. Purchases of poppy straw material were from 55.3 to 1,191.5 tons annual during years 1990 and 2006. The processing capacity of the Slovakian pharmaceutical industry is much higher, about 4,000 tons, and the miss raw materials are imported from Czech Republic. The cultivate varieties of poppy are suitable accumulate from 0.4 to 0.6% of morphine. However the purchase straw material contents in average only 0.3% of morphine, statistics from 1970 to 2005 years. The influence of the vegetation season and harvest technology is affected on these results.

Sm08.211

Phytochemical Characterization of Essential Oils from *Laurus nobilis* from Brazil and Turkey

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The aim of this work was to evaluate the yield and chemical composition of essential oil of laurel (*Laurus nobilis* L.) from Turkey and Brazil. Laurel leaves were collected at four properties in Paty do Alferes district (Rio de Janeiro State) in winter (07/26/2008), and dried at room temperature (25 °C) at shade conditions. Samples from Turkey were donated by a private company. Essential oils were obtained by hydrodistillation in a Clevenger-type apparatus for 4h and analyzed by GC-MS (Shimadzu, QP 5050, with DB-5 capillary column - 30 m × 0.25 mm × 0.25 µm). Carrier gas was Helium (1.7 mL/min); split ratio: 1:20. Temperature program: 60 °C, rising to 240 °C at 3 °C/min. Injector temperature: 240 °C and detector temperature: 260 °C. Identifications of chemical compounds were made by matching their mass spectra and Kovat's indices (IK) values with known com-

pounds reported in the literature. The average of essential oil yield obtained was 1.4% (Paty A and D samples), 1.5% and 1.1% (Paty B and C samples, respectively) and 2% (Turkey sample). Lower yield of Brazilian essential oils' may have occurred because the leaves were harvested in winter. Further studies will be conducted to verify the seasonal variation of laurel. Even smaller, they are within the acceptable market standard. Analysis by GC-MS of the essential oils has identified 16 compounds. The essential oil from Turkey presented a slightly higher content of 1.8 cineole (major compound), but it doesn't presented linalool, methyl eugenol and myrcene, compounds founded in Brazilian essential oils. These results showed the high quality of Brazilian essential oils' tested that indicates marketing potential to the consumer industry, without the onus of an import process. Brazilian and Turkey essential oils' presented similar quality and the first can supply the needs of internal Brazilian marketing, reducing costs of raw material and logistics.

Sm08.212

Eucalyptus from Mata Experimental do Escaroupim (Portugal): Evaluation of the Essential Oil Composition from Sixteen Species

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The introduction of *eucalyptus* in Portugal seems to have been part of a general movement, by the mid-nineteenth century, of ordering exotic plants to beautify parks and gardens. Nevertheless, given the favourable edaphoclimatic conditions and *Eucalyptus globulus* particular characteristics, this species was fast in becoming an unavoidable element of the Portuguese forest [1]. The Mata Experimental do Escaroupim (Salvatera de Magos, Portugal), is an area of protected forest tutored by Autoridade Florestal Nacional, which includes an arboretum with an identified, and documented, collection of 125 different eucalyptus species, considered to be the most complete in Europe [2]. In the present work, the essential oils isolated from the aerial parts of sixteen *Eucalyptus* species of this arboretum were studied. The essential oils were isolated and analyzed by GC and GC-MS as in [3], and the percentage composition of the volatiles was used to determine the oils relationship by cluster analysis [4]. Despite the monoterpene fraction being dominant in all oils (62-97%), major differences were found in essential oils composition. Essential oil cluster analysis showed only a high correlation (Scorr≥0.78) among eleven species (*E. cinerea*, *E. cordieri*, *E. bosistoana*, *E. botryoides*, *E. camaldulensis*, *E. globulus*, *E. polyanthemus*, *E. radiata*, *E. saligna*, *E. smithii* and *E. viminalis*), mainly due to their richness in 1,8-cineole (27-83%). The remaining five species were dominated by citronellal (36%, *E. citriodora*), piperitone (40%, *E. dives*), limonene and α-pinene (41% and 44%, respectively, *E. ficifolia*), α-pinene (82%, *E. pauciflora*) and α-phellandrene (45%, *E. urophylla*).

Sm08.213

Seed Germination and Dormancy Breaking Techniques for (*Echinacea purpurea* L. Moench)

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Echinacea purpurea (Asteraceae) is an important medicinal plant known as a disinfectant. Its germination percentage and germination rate is generally low due to dormancy. Dormancy and germination requirements were investigated in this plant. Seeds of *Echinacea purpurea* were subjected to different treatments including various levels of GA₃ (100, 200 and 300 ppm) and KNO₃ (0.5, 1 and 1.5 percentage) for 2 hours and prechilling (50 °C) for 1, 2, 3 and 4 weeks. The germination percentage and germination rate significantly increased in all of treatments. The highest germination and germination rate were obtained in prechilling treatment that induced over 90% germination. The mean germination time also improved in all of treatments and shortest mean germination time also was observed in prechilling treatment.

Sm08.214

Accumulation of Biologically Active Compounds in Above - And Underground Organs of Common Avens (*Geum urbanum* L.)

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Common avens is a perennial belonging to *Rosaceae* family. This species grows in Poland in deciduous and mixed forests on moderately humid and rich soils. Both above - and underground parts of this plant are rich in phenolic compounds, especially tannins. Underground organs contain also volatile oil with eugenol as a dominant compound. Raw materials of common avens reveal antidiarrheal, antihemorrhagic, antipyretic and antiseptic activity. Taking into consideration the high diversity of species the trials of its cultivation have been done. The aim of undertaken study was to investigate the influence of the age of plants and harvest time on the quality of above- and underground organs of common avens (*Geum urbanum* L.). In the raw materials the content of phenolic compounds (tannins and polyphenolic acids) and the content of eugenol and nopinone in essential oil from the roots were determined. In aboveground organs the content of tannins and polyphenolic acids was the highest in rosette leaves, lower in shoot leaves and the lowest in spring root leaves. Rosette leaves were characterized by high content of ellagic acid and epicatechin gallate. The content of tannins in underground organs was the highest in one-year old plants collected in late autumn. Two-year old underground organs were characterized by high content of catechin. Accumulation of eugenol and nopinone in essential oil from the roots was not related to the age of plants.

Sm08.215

Diversity of Wormwood (*Artemisia absinthium* L.) Growing Wild in Poland in Respect of the Content and Composition of Essential Oil and Phenolic Compounds

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Wormwood herb is regarded as a medicinal raw material rich in bitter principles. However, its biological activity is also affected by the presence of essential oil and phenolic compounds. In Poland wormwood herb is collected mainly from wild growing plants. The aim of the study was to compare the content and composition of essential oil and phenolic compounds in the raw materials obtained from eighteen populations of wormwood from central and north-eastern area of Poland. Herb was collected at the stage of plant blooming and dried naturally. Content of essential oil in the raw materials was determined by hydrodistillation method, and its composition by GC. Methanolic extracts obtained by continuous exhaustive extraction of raw materials were used for qualitative and quantitative analysis of phenolic compounds by HPLC. The content of essential oil in the investigated raw materials ranged from 0.40 to 0.88%. In six populations the dominant constituent of essential oil was sabinyl acetate, in three - chrysanthenyl acetate, in two - sabinene, and in one - beta-thujone. Other populations were characterised by comparable content of two or three main compounds in essential oil, e.g. sabinene and beta-myrcene, beta-myrcene and cineol, chrysanthenyl acetate and beta-myrcene, sabinyl acetate, chrysanthenyl acetate and beta-thujone. In the investigated raw materials eleven phenolic substances were identified: five phenolic acids (caffeic, p-coumaric, ferulic, chlorogenic, and rosmarinic one), five flavonoids (apigenin, diosmetin, orientin, quercetin, and hyperoside), and (-)-epigallocatechin gallate. In the raw materials obtained from all the investigated populations the dominant phenolic compound was ferulic acid but its content was diverse (396-1118 mg/100 g of dry herb). Remarkable level of chlorogenic and rosmarinic acids was also found (159-477 and 178-446 mg/100 g, respectively).

Sm08.216

Yield and Phytochemical Characterization of Essential Oil from *Ocimum selloi* B. Obtained by Hydrodistillation and Supercritical Fluid Extraction

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The yield and chemical composition of essential oils from leaves of *Ocimum selloi* B. submitted to organic and mineral fertilization, obtained by hydrodistillation and supercritical fluid extraction (SFE) were compared. Essential oil was extracted in a Clevenger-type apparatus for 2h 30 and analyzed by GC-MS (Shimadzu, QP 5050-DB-5 capillary column - 30m × 0.25mm × 0.25μm). Carrier gas was Helium (1.7 mL/min); split ratio: 1:30. Temperature program: 50 °C, rising to 180 °C at 5 °C/min, 180 °C, rising to 280 °C at 10 °C/min. Injector temperature: 240 °C and detector temperature: 230 °C. Identifications of chemical compounds were made by matching their mass spectra and Kovat's indices (IK) values with known compounds reported in the literature. An Applied Separations-apparatus (Speed SFE, model 7071, Allentown, PA, EUA) was used for SFE extractions. They were conducted at pressure 200 bar and temperature 30 °C (20 min in static mode and 40 min in dynamic mode). The supercritical CO₂ flow rate was (6.8±0.7) × 10⁻⁵ kg-CO₂/s. The essential oil collected was immersed in ethylene glycol bath (5 °C). The yield of essential oils obtained by SFE was larger than hydrodistillation in both fertilization treatments (279% e 333% for organic and mineral fertilizations, respectively). There were no differences between the fertilization treatments. The amount of the volatile components showed by GC-MS chromatogram was highest in the essential oil obtained by hydrodistillation than SFE. The main volatile constituents of the essential oils were trans-anethole (Hydrodistillation: organic - 52.4%; mineral - 55.0%/ SFE: Hydrodistillation - 62.8%; mineral - 66.8%) and methyl-chavicol (Hydrodistillation: organic - 37.3%; mineral - 38.3%/ SFE: organic - 8.4%; mineral - 4.3%). A reduction of methyl-chavicol relative proportion of essential oil obtained by SFE was observed. Cys-anethole, α-copaene, trans-cariofilene, germacrene-D, β-selinene, biciclogermacrene and spathulenol were expressed only in hydrodistillation. The extraction of essential oil by SFE presented larger yield of essential oil than hydrodistillation technique, presenting, however, these essential oils, different phytochemical profiles.

Sm08.217

The Effects of Postharvest Treatments on the Quality Parameters of Crude Drugs of *Thymus vulgaris* and *Origanum vulgare* subsp. *hirtum*

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The quantity and quality of the biologically active agents could be influenced significantly by the postharvest treatments. We aimed to compare the effect of natural drying, drying on 30 °C, on 40 °C, freeze drying (lyophilisation) and refrigeration on the quality of crude drugs of garden thyme (*Thymus vulgaris*) and Greek oregano (*Origanum vulgare* subsp. *hirtum*). The essential oil (EO) content and composition, the total antioxidant capacity (TAC) and the total phenol content (TPC) of samples were measured. The EO content of the thyme samples occurred between 0.68 and 1.85 ml/100 g d.w. The oregano samples contained EO within the range of 3.28 and 6.80 ml/100g d.w. The freeze drying significantly decreased the amount of EO in samples of both species. In the EO of Thymus samples thymol was found

in the highest ratio (58.7-71.8%), while in the essential oil of oregano carvacrol was the main compound (91.2-95.7%). The EO compositions were not influenced by postharvest treatments. Considering the TAC of the samples, only the water extract proved to be effective, while the alcoholic extracts had no TAC. Oregano has several times stronger TAC and TPC than garden thyme. Fresh and deep-frozen samples have not shown antioxidant capacity (0.00-0.21 mg ASE/ml). The strongest TAC was measured in case of natural drying (1.25 and 1.41 mg ASE/ml) and of 40 °C drying (1.03-1.42 mg ASE/ml) in the samples of both species. The TPC showed a little bit different character: In case of oregano naturally dried samples showed the highest values, while in case of thyme the freeze dried samples gave the highest values. Summarizing the results, deep freezing seems to have the worst effect on the quality parameters of the crude drugs.

Sm08.218

The Populations of *Melissa officinalis* L. (Lemon Balm) in Armenia and its Conservation through Introduction into Agricultural Production System

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It has been implemented field trips research work on the populations of *Melissa officinalis* L. in different regions of Armenia during 2008 - 2009 vegetation periods. Also, it has been surveyed its growing and biological characteristics in different populations. The selection of researching territories has been realized by taking into account the existing data (The Botanic Garden of RA) and theoretically possible existence of new population. These territories vary with their geographical location, elevation, soil types, climatic conditions etc. Though, we have found out new population, but the current condition of lemon balm's populations is alarming, due to many different anthropogenic threats. In fact, 4 populations from 11 are extinct. The map (with GPS data) shown the distributions of plant's populations is created. Also, it has been observed the factors, which have negative effect on the conservation of populations. Realized different multiple observations over the populations have exposed the fixed times of each phenophases, plant growing characteristics. We have estimated the growing sizes of each population, its abundance and the number of ripened plants etc. We have found out its certain biological characteristics in Armenia. *Melissa* is drought-resistant, but grows well in soils with enough moisture. It is susceptible towards early spring frosts and abrupt weather change. Chemical analyses have been done to value the essential volatile oils in plant, growing different populations. In order to contribute to the conservation of this plant, we suggest to introduce it into the agricultural production system. Created data could be served as indispensable materials for the appropriate approaches of domestication experiments of wild *Melissa*. This would foster the improvement of its use and the development of traditional medicine as well. In addition, created data could be basic material to assess the sustainability of *Melissa* populations in respect with The Red Book Criteria of IUCN.

Sm08.219

Morphological and Chemical Variability Assessment from Portuguese *Mentha pulegium* L. (Pennyroyal) Accessions

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Mint species have been used, for centuries, for medicinal and culinary purposes due to their antiseptic and cooling properties. *Mentha pulegium* L.; commonly known in Portugal as poejo, has local medicinal and seasoning use, namely in the preparation of typical fish dishes, and liquors. Accessions of *M. pulegium* consisting of wild collected material and seed progeny were assessed for diversity through a combined morphochemical evaluation. Morphological characters from ten, pheno-

lic constituents from eight, and essential oil yield and components from eighteen accessions were evaluated. The morphological characterization, used the *Mentha* descriptors, elaborated by Portuguese National Gene bank (BPGV), integrated in the ECPGR MAP WG (European Cooperative programme for Plant Genetic Resources Working Group on Medicinal and Aromatic Plants). Phenolics were identified and quantified by HPLC coupled with diode-array detection. The essential oils were isolated by hydrodistillation and analysed by GC and GC-MS, and the relationship between the different oil samples was assessed by cluster analysis. Morphological characters clustered the ten accessions in five groups, with six of them located in the main group, which was identified by low number of flowers in basal and terminal inflorescence and low pubescence in calix. Phenolic characters clustered the eight accessions in four groups. The phenol profile was the same in all accessions evaluated, but interpopulacional variability as observed, as well as with the morphological characters. The essential oil yield from the eighteen samples analysed ranged between 0.25-0.44% (v/f.w.). Oxygen-containing monoterpenes dominated all samples (96-97%), pulegone (65-87%), menthone (6-20%) and isomenthone (0.4-17%), being the main components. Cluster analysis confirmed a high chemical correlation among all accessions (Scorr≥0.97%) which is in agreement with previous studies on this species grown in Portugal, during the flowering phase and to data from other countries.

Sm08.220

Dropwort (*Filipendula vulgaris* L.) Seeds Germinability as Affected by their Ripeness and One-Year Storage

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Dropwort is a perennial, naturally occurring in Poland on sunny, semi-dry meadows and neglected lands. Above- and underground organs of this plant are used for ages in people medicine as a diuretic, antirheumatic and anti-inflammatory agents. In Department of Vegetable and Medicinal Plants of Warsaw University of life Sciences – SGGW the cultivation trials of dropwort have been done. The aim of undertaken investigation was to study the influence of seeds ripeness and its short term storage on the germination ability. The seeds were collected in the dough and complete mature stage from two-years old plants. The germinability was assessed directly after harvest and after one-year storage, according to The International Seed Testing Association (2008). The accumulation of phenolic compounds in the seeds was performed as well. Directly after seed harvest there were no distinct differences in germination ability between the seeds collected in dough and complete maturity. After one-year storage the germination ability of seeds collected in dough stage did not exceed 40 per cent whereas fully ripe seeds germination ability was higher than 90 per cent. In the seeds six phenolic compounds were identified: rutoside, hyperoside, astragalin, spiroside, gallic and ellagic acids. The distinctly higher germination ability of full ripe seeds after one-year storage may result in the decrease of the content of phenolic compounds, especially of gallic and ellagic acids.

Sm08.221

Intraspecific Variability of Southern Sweet-Grass (*Hierochloë australis* (Roem. & Schult.) Wild Growing in Poland

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Southern sweet-grass is naturally occurring in Poland tuft-grass. The leaves of this plant are coumarin raw material used in medicine as well as in food and tobacco industry. The content of coumarin in the leaves is the most important quality discriminant of the raw material. This species being under partial legal protection is seriously endangered because of excessive, uncontrolled collecting. Taking into

consideration this danger the trials of its cultivation were undertaken in our Department. The aim of the study was to investigate the intraspecific variability of southern sweet-grass wild growing in the Eastern area of Poland. The differences concerning the coumarin derivatives between five geographically distant populations and between 45 individuals (belonging to one population) were investigated. Both the populations and the individuals distinctly differed in the content of all detected coumarin compounds: coumarin, 3,4 - dihydroxycoumarin, o-coumaric acid. The highest differences between individuals were found in the content of coumarin (from 87.07 to 1082.43 mg/100g of dried raw material). There were also visible differences between individuals in the intensity of powdery coating on the leaves. The leaves of the plants rich in the coating were characterized by significantly higher content of coumarin in comparison with plants without visible coating. It may indicate that this compound is partly secreted from the leaves and accumulated in the powdery coating.

Sm08.222

Accumulation of Phenolic Compounds in Leaves and Underground Organs of Dropwort (*Filipendula vulgaris* L.)

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Dropwort is an Euro-Asiatic perennial belonging to *Rosaceae* family. The plant with shoots up to 80 cm high and pinkish-white flowers occurs rarely on sunny grass- or neglected lands. The raw materials (above- and underground organs) rich in phenolic compounds reveal anti-inflammatory, antirheumatic and diuretic activity. The effect of flowering shoots removal on the yield of basal leaves and underground organs (rhizomes with tuberous roots) and accumulation of phenolic compounds was investigated. The flowering shoots removal increased the yield of both above- and underground organs. In the plant materials six phenolic compounds [(+)-epigallocatechin, (+)-catechin, (-)-epicatechin, (-)-epigallocatechin gallate, ellagic and gallic acids] were determined by HPLC. In the leaves dominant compounds were gallic acid and (-)-epicatechin whereas in underground organs – catechin derivatives [(+)-catechin, (-)-epicatechin]. The summer removing of flowering shoots changed the content and composition of determined phenolic compounds. The storage organs (rhizomes and tubers) from the plants after flowering shoots removal were characterized by distinctly lower content of these compounds in comparison to raw materials from not-cut plants. The content of phenolic compounds in the leaves and roots of not-cut plants was higher than the plants after flowering shoots removal.

Sm08.223

Black Cohosh Rhizome and Phytochemical Production in Response to Shading, Spacing, and Age

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Black cohosh [*Actaea racemosa* L.; *Cimicifuga racemosa* (L.) Nutt.] is a perennial woodland herb native to the eastern and midwestern United States. Interest in cultivating black cohosh has increased as it is commonly used as a dietary supplement to treat menopausal symptoms. Two studies were conducted in a shadehouse in Missouri, USA from 2000 to 2004. The first study quantified the development of rhizome and root tissues of known age, and of four phytochemicals therein in response to three shading treatments. The second study evaluated rhizome and phytochemical yield in response to plant spacing and three shading treatments. In the age study, black cohosh rhizomes increased in size from of 46 g (fresh weight) at the end of their second season to 78 g by the third season. Mature rhizomes from the spacing study, which were several years old, averaged 171 g upon harvest. In

the age study, cimracemoside A, 23-epi-26-deoxyactein, actein R, and actein S were found in both rhizome and root tissues in varying quantities. In general, age of tissue did not consistently influence concentration of these phytochemicals. Rhizomes from plants in full sun had significantly more cimracemoside A (641 ppm) compared with those under 40% or 80% shade. For 23-epi-26-deoxyactein, rhizomes grown under 40% shade had a higher concentration (3,109 ppm) compared with 0 or 80% shade. In the spacing study, concentration of cimracemoside A was not affected by shading, but was significantly greater with 0.5 m spacing (427 ppm) compared with 1 m spacing; whereas concentration of 23-epi-26-deoxyactein was not influenced by spacing, but was significantly higher under 0% and 40% shade (2,842 and 2,779, respectively) compared with 80% shade. The varying responses of the phytochemicals in this study suggest that production of each compound may be independently influenced by a variety of horticultural and environmental factors.

Sm08.224

The Organic Cultivation of German Chamomile (*Matricaria chamomilla* L.)

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To study the effect of organic and bio fertilizers on the growth, yield and essential oil of *Matricaria chamomilla* L. a field experiment has been carried out in Iran during 2008. The factors were PGPR inoculation (inoculated and no inoculated), zeolite (0 and 9 ton/ha) and vermicompost (0, 5, 10 ton/ha). Studied characteristics were plant height, dry flower yield, essential oil content and yield. The results showed that vermicompost levels had significant effects on all studies parameters. With increasing vermicompost levels, plant height, dry flower yield, essential oil content and yield were increased. The highest (0.73 %) and the lowest (0.52 %) of essential oil content were observed in 10 and 0 ton/ha vermicompost levels, respectively. Also there were positive and synergistic interactions between factors vermicompost combined with PGPR and vermicompost combined with zeolite on dry flower yield. Zeolite also showed significant effects on mentioned traits as with increasing zeolite amount, essential oil yield increased and there was significant difference between 0 and 9 ton/ha zeolite. Also, PGPR enhanced plant height, dry flower yield, essential oil content and yield. The highest (358/13 kg/ha) and the lowest (321/33) of dry flower yield were obtained in inoculums and no inoculums PGPR. Consequently, it seems that the organic cultivation of German chamomile can consider as an alternative system for conventional system in chamomile production.

Sm08.225

Prioritisation of Medicinal and Aromatic Plant Species for Genetic Resource Conservation in Lithuania

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About 400 native plant species are being used in both modern and traditional medicine in Lithuania. They make an indispensable part of biological diversity and are to be conserved at large. As the number of the species is high and their habitat range is wide the application of maintenance measures on all of them gets complicated. Therefore, we made some attempts to prioritize the species and somehow facilitate the genetic conservation strategies for medicinal and aromatic plants (MAPs). For the baseline data collection we used national and regional pharmacopoeias, sci-

entific references, statistical and ethnobotanical data sources. The experience of neighbours, particularly the Nordic countries was taken into account as well. The analysis of different data sources and our own experience suggested grouping MAP species into three tentative priority groups based mainly on their socio-economic and cultural values, resource abundance, biological and habitat preferences. The socio-economic and cultural values of MAPs were estimated by their consumption rates and scale over the last period as well as species popularity and knowledge on it in different ethnographic parts of the country. The resource abundance and habitat preferences were estimated reviewing the various case studies as well as using inventory data of useful plants carried out by the Institute of Botany. The highest priority group includes MAP species which are (or could be) very important for the consumption, but their resources are very scarce due to specific habitat preferences and other conditions. The collecting of most of them is limited by the law. A good example of this group is *Arnica montana*. Lower priority group includes species of high utilitarian value with more abundant, but still limited resources. An example is *Arctostaphylos uva-ursi*. And the biggest part of MAP species which resources are not under the threat would fall into the lowest priority groups.

Sm08.226

Cholinesterase Inhibitory Capacity and Antioxidant Activity of *Quercus suber* L. Extracts

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Medicinal plant drug discovery continues to offer new and important leads against a variety of pharmacological targets including Alzheimer's disease (AD), which have a great impact on modern society. Currently, the effective treatment for AD has been aimed at the cholinergic system, via acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibition. Moreover, it has been suggested that oxidative stress is an important event to the development of multiple diseases including AD, and therefore, it seems reasonable that antioxidants can play an important role in their improvement. In this study, aqueous and methanol extracts from leaves of *Quercus suber* L.; a common tree of the Mediterranean region, were evaluated for their cholinesterase inhibitory capacity and antioxidant activity. The ability of the extracts to inhibit AChE and BuChE activity was determined by Ellman's colorimetric method. Antioxidant activity was evaluated by trolox equivalent antioxidant capacity (TEAC) and oxygen radical antioxidant capacity (ORAC) assays, and the phenolic content was evaluated by Folin-Ciocalteu assay. Results showed that the methanol extract of *Q. suber* was the most active inhibitor of AChE (37.31 - 89.05 %) and BuChE (30.20 - 68.75 %) enzymes *in vitro*. Both extracts demonstrated high antioxidant properties by TEAC (3576.08 ± 106.75 and 1892.48 ± 59.57 µmolTE·gext.⁻¹ for methanol and aqueous extracts, respectively) and ORAC (3071.80 ± 104.13 and 3624.00 ± 60.25 µmolTE·gext.⁻¹, respectively). Finally, it was confirmed a directly relationship between phenolic content (2945.53 ± 86.47 and 2535.30 ± 150.44 µmolGAE·gext.⁻¹ for aqueous and methanol extracts, respectively) and antioxidant activity, which are in accordance with previous studies. This study revealed that the methanol extract of *Q. suber* is a possible beneficial agent in AD therapeutic.

Sm08.227

Development of a Sustainable Method for the Bioprospection of Plumbagin from *D. intermedia*

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Plumbagin is a naphthoquinone produced by plants of the *Plumbaginaceae*, *Droseraceae* and *Drosophyllaceae* families, which has been known for long for its broad range of biological activities (antimicrobial, antiatherosclerotic, anticarcinogenic, etc) and continues to be an effective agent against new pharmacological targets. The most exploited source of plumbagin is the roots of *Plumbago* spp. (*Plumbaginaceae*), however these plants grow slowly and the roots suitable for extraction take years to grow. On the other hand, most species of the *Drosera* genus (*Droseraceae*)

and the monotypic species *Drosophyllum lusitanicum* (*Drosophyllaceae*) exist in confined habitats and are protected by law. Taking these limitations into account, this work describes the development of an alternative and sustainable method for the production of plumbagin from micropropagated *Drosera intermedia* (Hayne) plants. For the purpose of this work *D. intermedia* plants were produced using a recently developed micropropagation protocol. The biomass increment of *D. intermedia* cultures was monitored to determine the optimum subculture period and a growth curve was obtained. Several extraction techniques (maceration, ultrasound assisted, soxhlet and supercritical fluid extraction) were employed to recover plumbagin from the obtained plant material and extraction yields were compared. The crude extracts were then subjected to a purification step using a Solid Phase Extraction column. The results show that, except for maceration, good extraction yields were obtained and the purification process yielded a nearly pure sample. Furthermore, the use of supercritical fluids proved to be an efficient technique for the extraction of plumbagin, bypassing the need to use hazardous organic solvents. In conclusion, this work describes an ecologically and environmentally friendly method for the production of plumbagin.

Sm08.228

Sustainable Production of Basil Using Recycled Household Compost: Impact on Flavour and Quality

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Basil (*Ocimum basilicum* L.) is a commercially significant crop both in the UK and throughout the World. Since flavour properties of this herb are the primary factor determining its quality, our objective is to improve the sensory characteristics of the product, whilst improving sustainability by reducing energy and chemical inputs. Past research has shown significant phytochemical fluctuations in response to environmental stimuli, such as light, temperature and water. We hypothesised that the growth physiology and phytochemical content of basil is also influenced by nutrient levels and soil quality. We investigated the use of recycled household compost (RHC) as an alternative commercial substrate to soil-based media with the addition of chemical fertiliser. RHC is a widely-available waste by-product from domestic properties which may be beneficial to growers, the consumer and environment. Physiological measurements have been made and have shown that the use of RHC can result in a product with the required aesthetic qualities and yield for commercial use. We have established how the use of RHC substrate during cultivation affects the flavour characteristics of the plant, both via GC-MS analysis and human sensory evaluation.

Sm08.229

Water Extractable Phytochemicals from *Lavandula viridis* and *Thymus lotocephalus* Exhibit Antioxidant Properties

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It is well-known that most species from the *Lamiaceae* family possess a wide range of biological and pharmacological activities. *Lavandula viridis* and *Thymus lotocephalus* (*Lamiaceae*) are endemic species from the Algarve region (Portugal) that are little studied in terms of their pharmacological potential. Thus, and considering the increasing interest in the health properties of natural antioxidants, this study was conducted to ascertain whether the antioxidant activity and total phenolic content of extracts is affected by steeping the plant material in hot or cold water. The aqueous extracts were prepared by homogenizing aerial parts in cold water for 2 h or in hot water for 5 min (1:20 w/v). Both cold and hot infusions were then centrifuged at 3000 rpm for 10 min, the supernatant was filtered and used in subsequent assays. Antioxidant activity was evaluated by Fe²⁺ chelation, degradation of deoxyribose,

reducing of FeCl_3 and DPPH assays, and total phenol content was determined by using Folin-Ciocalteu reagent. Extracts from both species have similar iron chelating capacity and ability to inhibit $\text{Fe(II)/H}_2\text{O}_2$ -induced decomposition of deoxyribose as an index for hydroxyl radical. However, *L. viridis* extracts had higher reducing power than *T. lotocephalus* ones. Moreover, results obtained with DDPH and Folin assays also showed that extracts from *L. viridis* had superior antiradical properties and much greater phenolic contents than those from *T. lotocephalus*. In general, extracts obtained with hot water displayed higher antioxidant activity in all the assays and larger amounts of phenols, nevertheless, this was more pronounced in *L. viridis*. In conclusion, this work demonstrated the significant antioxidant properties of extracts from *L. viridis* and *T. lotocephalus* by employing several *in vitro* methods, however best results were observed with *L. viridis* extracts obtained with hot water.

Sm08.230

Introduction and Conservation of Mint Germplasm (*Mentha* spp.) in Brazil

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The genus *Mentha* (Lamiaceae) consists of nineteen species distributed in the Old and New World. The aromatic leaves of mint are used fresh and dried as flavorings or spices in a wide variety of foods. They contain biologically active constituents and are also used in traditional ceremonial rituals and as medicines. Volatile oils of mint species are used to flavor foods, in dental and oral products, and in fragrances. The objective of this work was to establish a germplasm bank of mint. The following species (Accessions) were included in this collection: *Mentha aquatica* L. (5); *Mentha arvensis* L. (6); *Mentha campestris* Schur. (1); *Mentha canadensis* L. (2); *Mentha* cf. *xgracilis* Sole (1); *Mentha* cf. *spicata* L. (6); *Mentha longifolia* L. (2); *Mentha x piperita* L. (12); *Mentha x piperita* subsp. *citrata* Ehrh. (2); *Mentha x villosa* Hudson (4); *Mentha rotundifolia* (L.) Hudson. (2); *Mentha* sp. (19); *Mentha suaveolens* Ehrh. (3); *Mentha suaveolens* Ehrh. x *M. aquatica* L. (1); and *Mentha sylvestris* L. (1). Sixty seven mint accessions, representing 14 species, were collected and introduced at the Embrapa Genetic Resources and Biotechnology, Brasília, DF, Brazil. The total germplasm collection is maintained at field and greenhouse conditions, as well as in *in vitro* collection. Most of the collection has been chemically characterized. Voucher of all the accessions have being collected and deposited in the Embrapa Genetic Resources and Biotechnology herbarium (CEN).

Sm08.231

Effect of Freezing and Drying on the Yield and Chemical Composition of Essential Oil of Rosemary (*Rosmarinus officinalis* L.)

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This work aimed to evaluate three post-harvest processes on chemical composition and yield of rosemary (*Rosmarinus officinalis* L.) essential oil. Leaves were collected at 8h30min in Jaguariúna-São Paulo State-Brazil. They were divided into three batches. The first batch was immediately submitted to hydrodistillation in order to know its initial volatile composition. The remaining batches were submitted to two different treatments. At one of these batches, the leaves were dried in a forced-air drier (35 °C/3 days). At the last treatment, leaves were placed in sealed, air-tight plastic food bags and stored at -20±0.5 °C for two weeks. Essential oils were obtained by hydrodistillation in a Clevenger-type apparatus for 2h30min and analyzed by GC-MS (Shimadzu, QP-5050, with DB-5-capillary column: 30 m x 0.25 mm x 0.25 µm). Carrier gas was Helium (1.7mL/min); split ratio: 1:20. Temperature program: 60 °C, rising to 240 °C at 3 °C/min. Injector temperature: 240 °C and detector temperature: 260 °C. Identifications of chemical compounds were made by matching their mass spectra and Kovat's indices (IK) values with known compounds reported in the literature. The data of yield and chemical composition were submitted to ANOVA, followed of Tukey's test. The highest essential oil con-

centrations were observed in frozen samples (0.94%-a), followed by dried samples (0.92%-b). Essential oil from fresh-material presented smaller yield (0.81%-c). Comparison of the essential oil profiles showed no statistically significant differences in camphor content (fresh-26.9%-a; dried-26.9%-a; frozen-25.7%-a), major component. The compounds, 1,8 cineole (fresh-19.9%-a; dried-18.5%-a; frozen-14.4%-b) and myrcene (fresh-15.9%-a; dried-15.7%-a; frozen-11.9%-b), showed highest content on fresh and dried samples. α -pinene (fresh-14.4%-b; dried-13.8%-b; frozen-16.6%-a), camphene (fresh-4.0%-b; dried-4.3%-b; frozen-5.1%-a) and α -limonene (fresh-3.9%-b; dried-4.1%-b; frozen-4.9%-a) presented highest content when submitted to freezing. These results showed that freezing process increased the yield of rosemary essential oil, but changed its composition, but, for some aromatic species, a proportion of the oil yield must be sacrificed to ensure the required essential oil quality.

Sm08.232

Effect of Vermicompost, PGPR and Zeolite Application on Yield and Essential Oil Content of Anise (*Pimpinella anisum* L.)

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In this research, the effects of bio-organic fertilizers and zeolite on seed yield, essential oil content and composition of *Pimpinella anisum* L., as, aromatic and medicinal plant, was investigated. Field experiment was conducted in the Agriculture Research Station at Sanandaj, Iran, in 2008. Experimental Factors were three levels of vermicompost (0, 5 and 10 t-ha⁻¹), two amount of zeolite (0 and 4.5 t-ha⁻¹) and mixture of *Azotobacter*, *Azospirillum* and *Pseudomonas* (inoculation and not-inoculation). The results showed that vermicompost levels highly increased the quantitative characters including plant height, umbel no/plant and seed yield. The application of 10 t-ha⁻¹ vermicompost gave the highest value of those characters. These values were 18.3%, 29.9% and 43.1% higher than the first level of Vermicompost, for plant height, umbel no/plant and seed yield, respectively. Quantitative characters were also significantly increased by PGPR inoculation and zeolite application. Applying the third level of vermicompost caused the highest value of essential oil and anethol percentage. Zeolite application had a significant effect on essential oil content, but no significant effect on anethol percentage. Generally the most favorable interaction treatment for umbel no/plant and seed yield was the PGPR inoculation combined with the highest level of vermicompost.

Sm08.233

Comparison between Different Techniques for Volatiles Analyses in Coriander (*Coriandrum sativum* L.)

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Although many papers have been devoted to the study of Coriander fruits aromatic pattern, many differences show up according not only to the provenience of seeds, but also to the analytical methods chosen. In our work, samples from one Coriander small-seeded biotype have been analysed, verifying the differences between (1) the oil extracted by means of a Soxhlet apparatus (2) the same oil sampled by means of HS-SPME and (3) the HS-SPME on the roughly ground seeds, all analysed by direct injection in the GC-MS. The volatile composition detected by means of HS-SPME on oil resembles tightly that performed on Soxhlet extract. With respect to the direct injection, the HS-SPME shows a higher sensitivity towards the less heavy and therefore more volatile compounds. The HS-SPME on roughly ground seeds is able to highlight the more volatile compound as limonene, gamma-terpinene and in particular the E-2-decenal that in hot extraction is irretrievably lost.

Sm08.234

Cultivation Trials of Dill (*Anethum graveolens* L.) with Different Row Arrangements

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Dill (*Anethum graveolens* L.) is an annual plant from *Umbelliferae*. Although in cookery also sprouts and tender leaves are used, the drug from Dill is represented by the scented fruits ("seeds"), largely used for flavouring conserved foods and liqueurs, but also by herbal and cosmetic industry. In this work, we present the results of a trial performed in Sicily, putting under comparison four different row arrangements: CR30 (continuous rows 30 cm apart); CR60 (continuous rows 60 cm apart), CR90 (continuous rows 90 cm apart) and TR (twin rows with a distance of 30 cm inside and 60 cm between twin rows. Being constant the plant population on row (12 plants·m⁻¹), the plant density per unit area changed consequently to 40, 20, 13.3 and 26.6 plants·m⁻², respectively. Seeds yield, ranging from more than 2 to less than 0.8 t·ha⁻¹, seemed to vary according to plant population, but between treatments many differences showed up in yield components and plant geometry.

Sm08.235

Effects of Nutrients on Growth and Active Substances of Lemon Balm (*Melissa officinalis* L.)

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A greenhouse experiment was conducted to evaluate the effects of nitrogen, phosphorous, potassium and a complete fertilizer (Agrihansa) on growth and active substances of seedlings of lemon balm (*Melissa officinalis* L.). The plants were transplanted in pots containing 1/3 soil, 1/3 sand and 1/3 peat (v/v) at 4-6 leaf stage and kept in a greenhouse. The treatments were using N, P, K, NP, NK, PK, NPK and Agrihansa and comparing them to control (without using fertilizers). N, P₂O₅ and K₂O were applied at a concentration of 50 mg/kg of pot soil. The results indicated that maximum shoot fresh (141.57 g/plant) and dry (37.21 g/plant) weights were observed in NP treatment. The maximum shoot height (41.85 cm) was achieved on PK treatment which was not significantly different when compared to NP, NK and Agrihansa. The highest green herb (109.07 g/plant) and drug herb (27.34 g/plant) weights were obtained in NP treatment. Drug leaves weight (16.28 g/plant) had the highest amount in NP treatment. The maximum essential oil percentage of drug leaves (0.4%) was obtained in P treatment which was not significantly different when compared to N, K, NP and control. The highest essential oil content of drug leaves (64 mg/plant) was achieved when NP was used. Qualitative analysis of essential oil by GC/MS led to identification of 20 components. The major components were geranial and neral. The highest quantities of geranial (45.33%) and neral (34.1%) were observed in NP and N treatments, respectively.

Sm08.236

Aromatic Plants and their Bioactive Products to Control Postharvest 'Rocha' Pear Diseases

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Aromatic plants can play a determinant role in several domains of agriculture being recognized as useful for food preservation, preventing moulds and pest attacks that affect the quality of fresh fruits. Post harvest deterioration of fruits due to water loss, senescence and development of physiological disorders and fungal attack cause decay in quality and economic losses. In present work the extracts of

a set of aromatic plants were assayed for their capability to control fungi affecting cold stored fruits. Assays were performed *in vitro* and *in vivo* using the 'Rocha' pear variety kept under cold chambers. *Botrytis cinerea* and *Penicillium expansum*, two main pathogens responsible for postharvest rots, were used as biological targets. Although the low concentrations used promising results were achieved, from the *in vitro* tests performed with extracts or oils of eight aromatic plants. Significant mycelia growth reduction of both fungi was obtained mainly with *O. vulgare*, *Coriandrum sativum* and *Mentha pulegium*. Successful *in vivo* assays were performed with *O. vulgare* either for preventing or to treat fungal infections. From tests performed with standard compounds corresponding to the main constituents of such aromatic plants, positive results were also obtained mainly with caprylic acid, benzoic acid and pulegone. Present work is a positive contribute to the knowledge on biological potentialities of aromatic plants and their helpfulness to the development of harmless strategies to post-harvest control of fruit rots.

Sm08.237

Agronomic and Chemical Characterization of Vetiver Accessions

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Vetiver (*Chrysopogon zizanioides* (L.) Roberty syn. *Vetiveria zizanioides* (L.) Nash) is a perennial plant of the family *Poaceae* whose essential oil extracted from its roots have jobs defined in the perfume industry because its known natural features such as fixation of volatile formulations. The aim of this study was to realize agronomic and chemical characterization of the vetiver accessions of the Active Germplasm Bank of the Federal University of Sergipe for identification of highly productive clones for essential oil content, yield and chemical composition. We tested six clones (UFS-VET001, UFS-VET002, UFS-VET003, UFS-VET004, UFS-VET005 and UFS-VET006) in a randomized block design with three replications and each part consisted of three vessels of 30 dm³ with one plant. After six months of culture the following variables were evaluated: plant height, number of tillers and ahrs, dry weight of shoot and root, essential oil content and yield, and chemical composition of the essential oil. The increased production of root biomass (1016.80 g) was obtained by accession UFS-VET003. The accessions UFS-VET002 and UFS-VET003 showed the highest percentage of khusimol (24.21% and 22.25%, respectively), the principal chemical constituent for the commercial value of vetiver essential oil.

Sm08.238

Genotype - Age Interaction in Pepper-Rosmarin

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This work was aim to study the behaviour of pepper-rosmarin (*Lippia sidoides* Cham) accessions of the Active Germplasm Bank of the UFS. The randomized block design was used in a split plot in time scheme. We tested ten pepper-rosmarin accessions and we realized harvests in the rainy seasons of 2005 and 2006. We analyzed the following variables: plant height (cm), fresh and dry weight of leaves (g/plant), content (%) and yield (mL/plant) of essential oil, and content (%) of the chemical constituents of the essential oil. We observed highest content and yield of essential oil for accession LSID-105 with 7.68% in 2005 and for accession LSID-102 with 56.46 mL/plant in 2006. The highest thymol and carvacrol means were found by accession LSID-003 with 90.82% and accession LSID-104 with 56.05%, respectively, both in 2006. The accession LSID-104 collected in the Sergipe State, presented carvacrol as its major chemical constituent. The other accessions had thymol as their major chemical constituent. In both years of cultivation the accessions presented phenotypic variability for all the analyzed variables, except for terpin-4-ol content in the essential oil.

Sm08.239

New Aromatic *Citrus* Resources in Okinawa

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Okinawa Islands (Japan), which lie between 24° and 27°N, have long been an important trading zone connecting East and Southeast Asia. Their subtropical climate differs from those of other citrus production areas in Japan, and is more like that of Taiwan or the northern Philippines. The islands grow many kinds of subtropical and tropical plants, including *Citrus* varieties that are commonly used for medicinal purposes in Okinawa, China, India, and Southeast Asia. We have explored these *Citrus* genetic resources and analyzed their functional possibilities, including those of their aromatic components, since 2008. Most *Citrus* in the Okinawa Islands are categorized into the mandarin group. The biggest species group, *C. depressa* Hayata, produces small mandarins with a strong, fresh aroma that are used as sour or as sweet citrus. This group has many variations, and fruits contain high amounts of flavonoids. We have identified several *Citrus* species in the Okinawa Islands that have unique aromatic profiles that could find use in aromatherapy, cosmetics, and other applications. Four unique *Citrus* profiles are introduced here. 1) *Citrus depressa* Hayata (local name Shiikuwasha): A small mandarin with a high content of the monoterpene hydrocarbons γ -terpinene and p-cymene. Fruit weight 30–40 g. Fruit shape index (FSI)* = 137. Peel is vivid orange yellow and 1.5–2.0 mm thick, and contains polymethoxyflavones (nobiletin, tangeretin) and a flavanone (hesperidin). The essential oil could be used in cosmetics, aromatherapy, and foods. 2) *Citrus keraji* hort. ex Tanaka var. *kabuchii* (local name Kabuchii): High in sesquiterpene hydrocarbons and thymol, with a strong, fresh aroma. The essential oil could be used in aroma treatment for relaxation and refreshment, as a room fragrance and deodorizer, in cosmetics, and in foods and drinks, especially alcoholic beverages. Fruit weight 75–85 g. FSI = 127. Peel is bright yellowish-green and 3–5 mm thick. The essential oil yield is higher than that of *C. depressa*. 3) *Citrus keraji* hort. ex Tanaka (local name Keraji): High percentages of esters (geranyl acetate and neryl acetate), similar to bergamot, make it potentially good for relaxation in aromatherapy. This species could become a new Japanese bergamot adapted to high humidity. Fruit weight 75–85 g. FSI = 113. Peel is dark yellowish-green and 4–5 mm thick, with a favorable aroma. 4) *Citrus* spp. (local name Unzohki): High percentages of geraniol and nerol make it good for cosmetics and aromatherapy treatment. Fruit weight 100–120 g. FSI = 121. Peel is vivid orange-yellow and 6–10 mm thick, with a fresh citrus aroma like *C. nobilis* Lour. These aroma profiles suggest the potential for further development of essential oil production and utilization of wastes from juice factories in Okinawa. In addition, the high flavonoid contents of these fruits could allow the development of human health care applications.

Sm08.240

Influences of Artificial Lightweight Soils and Soil Moisture on the Growth of English Lavender and Spearmint in Rooftop Gardening

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Rooftop greening is expected to reduce the heat island phenomenon by relieving the outdoor thermal environment and to function to protect insulated buildings. However, we sometimes observe poor growth or the death of rooftop herbs on account of inappropriate watering. In this experiment, seedlings of English lavender (*Lavandula angustifolia*) and spearmint (*Mentha spicata*) were planted in small nursery boxes (L240 × W160 × H70 mm) filled with four types of artificial lightweight soil. Controlled soil moisture was of five levels. Watering was carried out when soil moisture potential in the nursery boxes reached 12, 34, 56 and 78% of field water capacity (100%) respectively, to bring them back to 100%. In addition, soil moisture potential was maintained at 100% in one treatment. Differences with respect to the artificial lightweight soils were observed in the growth of lavender and mint. However,

greater than these differences was the difference in soil moisture among the artificial lightweight soils. A cross-soil moisture comparison of plant height, number of leaves, and top fresh weight of the lavender yielded the following figures: 78% > 56% > 34% > 12% ≥ 100%. The ratio of plant height, number of leaves, and the fresh and dry weights of the top at 100% of soil moisture to those at 78% of soil moisture was 68, 43, 37 and 41%. Essential oil content in the lavender leaves was also higher at 78% of soil moisture than at the other treatments. As for spearmint, plant height, number of leaves, and top fresh weight tended to increase with the increase of soil moisture percentage, especially at 100% of soil moisture. Also, a marked defect in growth was observed at 12% of soil moisture. Essential oil content in the spearmint leaves was higher at 12% of soil moisture than at the other treatments.

Sm08.241

Medicinal Pastures: Germination Assessment of Chicory (*Cichorium intybus*) Seeds Collected in Alentejo, South Portugal

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In modern sustainable grazing systems, there is increased emphasis on use of non-traditional forages and local varieties to supply nutrients to livestock. Low availability of forage early in the growing season and low nutritive value in the summer could limit animal production in Alentejo. Chicory (*Cichorium intybus*) is perhaps best known for the extract of its roots used as an ingredient in 'coffee substitute' beverages or even for its medicinal uses. The plant blue flowers also present ornamental potential. Its use as a grazed forage for ruminants is less well known. Several authors reported its high content of some major and minor trace minerals in chicory and commented on its use in pasture mixtures as a source of these minerals. Others reported its potential use as medicinal pasture, with positive effect as a dewormer. The main goal of these studies is to assess local populations of *C. intybus* aiming its propagation to use as grazed forage with medicinal purposes in Alentejo and in the Mediterranean basin. *C. intybus* seed quality depends on the genetic characteristics of the plants, but it is also strongly affected by the conditions of seed development on the mother plants and by storage conditions. Seeds didn't show dormancy, germinating in a large range of temperatures (5 ° to 30 °C), with its optimum around 25 °–30 °C. Seeds from 10 accessions of *C. intybus* were collected in the Alentejo region in beginin of autumn 2009 and stored at room temperature (ca. 20 °C). Seeds were chilled at 5 °C for 15 days or stored at room temperature (20 °C) (control). Germination trials were performed in light and temperature controlled incubations at 20 °C at total darkness and at 12-h daily photoperiod.

Sm08.242

Study of Chemical Composition and Yield of Essential Oil of *Ocimum selloi* B. Submitted to Hydrodistillation and Steam Distillation

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The present study aimed to evaluate the yield and chemical composition of essential oil of *Ocimum selloi* obtained by steam distillation and hydrodistillation. The leaves were collected on experimental area of Embrapa Environment (Embrapa Meio Ambiente - Jaguariúna -SP/ Brazil) and dried at 35 °C for three days. The essential oil was obtained by hydrodistillation in a Clevenger-type apparatus and steam distillation apparatus for 2h30. The yield was calculated using the essential oil mass values. Essential oils were analyzed by GC-MS (Shimadzu, QP 5050-DB-5 capillary column - 30 m x 0.25 mm x 0.25 µm). Carrier gas was Helium (1.7mL/ min); split ratio: 1:30. Temperature program: 50 °C, rising to 180 °C at 5 °C/ min, 180 °C, rising to 280 °C at 10 °C/ min. Injector temperature: 240 °C and detector temperature: 230 °C. Identifications of chemical compounds were made by matching their mass spectra and Kovat's indices (IK) values with known compounds reported in the literature. The essential oil of *Ocimum selloi* did not presented differences in number of compounds. Were identified the fol-

lowing compounds: Cys-beta-ocimene, methyl chavicol (major compound), alpha-copaene, beta-bourbonene, beta-elemene, methyl eugenol, trans-caryophyllene, trans-alpha-bergamotene, allo-aromadendrene, germacrene-D, beta-selinene, bicyclogermacrene, germacrene-A and delta-cadinene. There was a reduction on methyl chavicol content on essential oil extracted by steam distillation (86.6%) when it was compared to hydrodistillation (93.2%). The inverse was noticed to bicyclogermacrene that presented higher concentration when it was extracted by steam distillation (2.6%) than hydrodistillation (1.9%). There was significant loss of essential oil during the extraction process by steam distillation (approximately 25% lower). These results showed that hydrodistillation presented highest yield of essential oil and highest content of methyl chavicol, when it was compared to steam distillation. At the condition in which the experiment was performed, hydrodistillation is the extraction form more suitable for essential oil of *Ocimum selloi*.

Sm08.243

Mountain Arnica Transplants Production by Usage of Mycorrhizal Fungi

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Medicinal plant species mountain arnica (*Arnica montana* L., family *Asteraceae*), a herbaceous plant of European hilly-mountain region, grows on acidic lawns and dry heather-lands, and is present in plant community Arnico-Nardetum strictae in western part of Croatia. Negative changes in eco-system (air pollution, over-fertilization), abeyance of pasture and mowing of lawns, and excessive gathering from the nature, lead to continuous decline of natural populations, proclamation of species as vulnerable and protecting by law in many European states, including Croatia. The aim of research is introduction of arnica into agricultural production on agro-ecologically suitable lands (hilly region, acidic soils. with low nutrient content), along with diminishing of plant mortality, especially expressed in the first year of cultivation. It is presumed that plant mortality could be decreased by using of mycorrhizal fungi of the genus *Glomus* and/or *Trichoderma* in transplants production. Transplants were grown in PP trays with 67 cells, 9 cm deep, filled with commercial organic substrate inoculated with 5 % inoculum of *Glomus mosseae* (GM) and *Glomus intraradices* (GI), pellets of *Trichoderma harzianum* (TH, one pellet per cell) and in untreated substrate as control (C). The trial was set up in the summer growing period, in the open space with net-protection, according to complete randomized block with four replications. Since initial growing was very slow, seedlings were pricked out two months after seeding into 11 cm deep pots, and transplanted three weeks later in agricultural field, according to Latin square with four replications. Growth parameters (leaf rosette diameter, number of leaves per plant, plant biomass) analysed before pricking and planting out, and six weeks after planting, showed significant positive effect of TH on growth and development of arnica plants, as well as negative influence of GM and GI.

Sm08.244

Seed Germination Behaviour of the Endangered Medicinal Plant *Podophyllum hexandrum* Royle

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The overexploitation of *Podophyllum hexandrum*, a Himalayan medicinal species belonging to *Berberidaceae*, is endangering its survival in natural sites. Etoposide, a derivative of the podophyllotoxin, is currently in clinical use for treatments against many cancers. The cultivation of this species may answer to the growing demand of this plant species and may assure its supply. However, the difficulties in seed germination have not favored the cultivation of this plant. Several studies on this

subject were published without giving a final answer. Germination tests started in 2008. Seeds were collected from a culture in Switzerland in August 2008, washed and dried, then conserved at room temperature. From the harvest on and during 200 days, germinations were made 13 times at regular intervals. Seeds treated with gibberellic acid GA3 (450 ppm, 24h) were compared to untreated seeds (in Petri dishes in a germinator with 12 hours with light at 25 °C and 12 hours in the dark with 18 °C). The development of excised embryos were also analysed in a similar way on the Gamborg B5 medium with or without GA3 (2.5 µmol/l). More than 90% of the excised embryos presented a normal growth from 5 to 8 days after having been put in culture. No behaviour difference was observed between the embryos issued from fresh seeds at harvest and those issued from dried seeds up to 12 weeks after harvest. The addition of gibberellic acid to the culture medium had no effect on the growth rate or speed. The best result for seed germination was obtained by treating fresh seeds at harvest with GA3, allowing 90 % germination after 80 days. Complementary tests including the importance of temperature on germination rate and duration will be necessary to better define the procedure to get plants from seeds for cultivation of *Podophyllum hexandrum*.

Sm08.245

Variations in Terpene Profiles of Different Strains of *Cannabis sativa* L.

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Secondary compounds of the plant are indispensable to cope with its often hostile environment and the great chemical diversity and variability of intraspecific and interspecific secondary metabolism is the result of natural selection. Recognition of the biological properties of secondary compounds have increased their great utility for human uses; numerous compounds now are receiving particular attention from the pharmaceutical industry and are important sources of a wide variety of commercially useful base products. Medical and other effects of *Cannabis sativa* L. are due to concentration and balance of various active secondary metabolites, particularly the cannabinoids, but including also a wide range of terpenoids and flavonoids. Literature reported a wide qualitative and quantitative variability in cannabinoids, terpenoids and flavonoids contents in *Cannabis* spp. Terpenes are strongly inherited and little influenced by environmental factors and, therefore, has been widely used as biochemical marker in chemosystematic studies to characterize plant species, provenances, clones and hybrids. Aim of this study was to investigate the variability in terpene profiles in *Cannabis sativa* L. Samples were collected from 16 inbred lines derived from 16 different strains and were dried at room temperature. The terpene composition in inflorescences was analysed by GC/FID and GC/MS. The amount of each terpene (in sufficient quantities to be considered in statistical analysis) was expressed as a percentage of total terpenes. Results showed a large variation between different strains in the relative contents for several monoterpenes (α -pinene, camphene, β -pinene, sabinene, Δ -3-carene, α -phellandrene, β -myrcene, α -terpinene, limonene, 1.8-cineole, γ -terpinene, cis- β -ocimene, trans- β -ocimene, α -terpinolene) and one sesquiterpene, β -caryophyllene. This wide variability in terpene composition can provide a potential tool for the characterization of *Cannabis* biotypes, and warrant further researches in order to evaluate the drug's medical value and, at the same time, to select less susceptible chemotypes to the attack of herbivores and diseases.

Sm08.246

Study of Chemical Composition and Antioxidant, Antibacterial and Cytotoxic Activities of *Salvia urmiensis* Bunge from Iran

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The genus *Salvia* (*Lamiaceae*) comprises more than 700 species and wide spreads

over the world. In Flora Iranica, this genus is represented by 58 species, 17 of which are endemic. The genus *Salvia* is known for medical value, antibacterial and antioxidant properties. *S. urmiensis* was collected during the flowering stage from Azerbaijan Garbi Province, NW Iran. The aerial parts were hydrodistilled for 6 hours, using a Clevenger-type apparatus to yield 0.5% of dark yellowish oil. The oil was analyzed by GC-MS. 50 compounds were characterized in the essential oil of *S. urmiensis*, which 5-(7a-Isopropenyl-4,5-dimethyl-octahydroinden-4-y (19.149%), Sclareoxide (Cis-A/B) (18.360%), (+) Spathulenol (15.565%), Sclareolide (6.992%), 13(16),14-Labdien-8-ol (5.671%) and Sclareol (5.671%) were found to be the major components. Total phenolic content in the extract was 0.27 mg Gallic acid equivalents (GAE)/g and total flavenoid content was 11.32 mg/g (DW). Antioxidant activity (IC₅₀ value) of the extract was determined as 1.51 mg/ml by DPPH assay. The methanolic extract showed a high antibacterial activity against the bacteria *Bacillus subtilis* and *Enterobacter aerogenes*. It showed a moderate activity against *Serratia marcescens* and *Pseudomonas aeruginosa* and a low activity against *Escherichia coli*, *Bacillus megaterium* and *Bacillus cereus*, but it has no activity against *Staphylococcus aureus*, *Staphylococcus saprophyticus* and *Citrobacter amalonaticus*. In addition, the methanolic extract showed cytotoxic effect against two cancer cell line (Breast Cancer MCF-7 and Colon Cancer HT-29).

Sm08.247

Analysis of Chemical Composition, Antioxidant Potency and Antibacterial Activity of *Salvia staminea* Montbr. & Auch Ex. Benth. from Iran

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The genus *Salvia* (Lamiaceae) with more than 700 species widely distributed over the world. In Flora Iranica, this genus is represented by 58 species, 17 of which are endemic. The genus *Salvia* is known for medical value, antibacterial and antioxidant properties. *S. staminea* was collected during the flowering stage from Mahanian, Qazvin Province, W Iran. The aerial parts were hydrodistilled for 6 hours, using a Clevenger-type apparatus to yield 0.5% of dark yellowish oil. The oil was analyzed by GC-MS. 50 compounds were characterized in the essential oil of *S. staminea*, which α -Copaene-8-ol (15.10%), Germacrene-D (12.258%), α -Pinene (11.045%), β -Pinene (9.44%), Bicyclgermacrene (7.322%) and 4-Formyl-3,5-di-*t*-butylbenzoic acid (3.040%) were found to be the major components. Also total phenol content in the extract was 1.69 mg Gallic acid equivalents (GAE)/g and total flavenoid content was 12.15 mg/g (DW). Antioxidant activity (IC₅₀ value) of the extract was determined as 1.55 mg/ml by DPPH assay. In addition, the methanolic extract showed high antibacterial activity against the bacteria *Bacillus cereus* and *Bacillus subtilis*. It showed moderate activity against *Bacillus megaterium*, *Enterobacter aerogenes*, *Serratia marcescens* and *Pseudomonas aeruginosa* and low activity against *Escherichia coli* but it has no activity against *Staphylococcus aureus*, *Staphylococcus saprophyticus* and *Citrobacter amalonaticus*.

Sm08.248

Comparative Studies on the Polysaccharide and Amino Acid Content in Natural and Cultivated *Poria cocos*

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Poria cocos (Schw.) Wolf is a well-known traditional Chinese herbal medicine that has been used as a diuretic, sedative and tonic drug. However, the natural *P. cocos* forms sclerotia on the roots of cedar or pine, its productivity is limited. Therefore, it is imperative to find a cultivated *P. cocos* substitute for the natural one. The aim of this study is to establish theoretical foundation for the species domestication by the determination of Polysaccharide and amino acid. The molecular marker SRAP (Sequences-related Amplified Polymorphism) was applied to discriminate natural *P. cocos* 28 and cultivated *P. cocos* 578. The Phenol-sulfuric acid procedure

and amino acid standard analysis method are used to the determination of Polysaccharide and amino acid. The results demonstrated that different bands were found in 3 SRAP primer combinations out of 26 ones and plentiful Polysaccharide and amino acid were detected in *P. cocos* 28 and 578, 19.33%, 17.67% and 15.116 %, 15.053 %, respectively. Both *P. cocos* contain essential amino acid except for Trp (Trp was totally destroyed by Hydrochloric acid hydrolysis method). The ratio of essential amino acid to total amino acid in cultivated *P. cocos* 578 reached 60.85%, litter higher than that of natural *P. cocos* 28, 59.07%. Besides this, the content of Ser, Glu, Gly, Val, Phe in cultivated *P. cocos* 578 are higher than that of the natural *P. cocos* 28. Therefore, the cultivated *P. cocos* 578 could be the alternative strain of natural *P. cocos* 28. The study indicated that SRAP marker could be an effective molecular marker to the analysis of genetic diversity of *P. cocos*. The information illustrated by this study is useful for selecting high-yield cultivation of *Poria cocos*.

Sm08.249

Leaf Biomass, Yield and Content of Saffrole in *Piper aduncum* L. Essential Oil in Different N Levels and Harvest Ages

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The objectives of this study were to assess the influence of five doses of N in leaf biomass production, income and level of saffrole in *Piper aduncum* L. essential oil in four harvest ages. The statistical design was randomized block with 5 rates of N and four harvest dates, in a 5 x 4 factorial scheme with four replications. Nitrogen levels were 0, 45, 90, 135 and 180 kg-ha⁻¹. The harvest ages were 152, 242, 332 and 428 days after transplanting (DAT). The extraction of essential oil of leaves was done by hydrodistillation, and the saffrole content was determined by gas chromatography coupled to mass spectrometer. The data were submitted to analysis of variance and compared by the Tukey test at 5% of probability. We observed a linear increase in biomass production according days after transplantation, and the highest production occurred at 180 kg-ha⁻¹ for the last three harvests. The highest essential oil content (%) occurred at 152 DAT, with the 332 and 428 DAT in doses of 135 and 180 kg-ha⁻¹. We also observed a linear increase in the essential oil yield (g/6 plants), and for the last two harvests was higher at doses of 135 and 180 kg-ha⁻¹. The nitrogen and harvest ages did not affect the content of saffrole present in the essential oil, however, the yield of saffrole was significantly higher in the last harvest and in the two highest doses of N.

Sm08.250

Prevalence and Severity of Geranium Wilt Complex Disease in Garhwal Hills of Uttarakhand in India

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Geranium (*Pelargonium graveolens* L. Herit) is a source of medicinally and industrially important essential oil extracted from its leaves and tender shoots. Species is becoming favoured plantation crop due to its adaptability to low and mid hills of Garhwal at altitudes ranging from 1500-2100 m asl. During a survey of geranium fields in Garhwal, the crop was found suffering from a disease exhibiting symptoms of root rot and wilt complex causing considerable damage (about 70%) to the crop. The pathogens were identified as *Rhizoctonia solani* Kuhn and *Fusarium solani* and further confirmed by ITCC, IARI, New Delhi (ITCC Ref. No. 6199 and 6200). The extensive survey for disease was carried out in the year 2005 to 2007 during summer to winter season in randomly selected fields in four district viz.; Tehri, Uttarakashi, Dehradun and Chamoli of Garhwal hills. The summer and winter planted crops of 2005, 2006 and 2007 were found to be severely affected by stem and root rot wilt. Plants showed maximum stem rot incidence (60.55%) and plant mortality (17.20%) during the winter season, whereas in summer the crops showed maximum root rot and wilt complex incidence (38.33%) with plant mortality up

to 12.22%. The average stem rot mortality was comparatively low (16-20%) in summer crop but increased 18 to 26% in winter crop. The percentages of root and stem rot mortality in plantation of were initially low but had increased to 37.35% (summer crop) and 26% (winter crop) at Ranichauri.

Sm08.251

The *ex situ* Comparison of Two Improved St. John's Wort (*Hypericum perforatum* L.) Cultivars with an Iranian Wild Population

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In this research, two improved cultivars of *Hypericum perforatum* ('Gold' and 'Veperikon') were compared with a wild Iranian population (*Ardabile population*) under common garden conditions in Iran. Plants were cultivated from seed in a greenhouse and seedlings were transplanted after one month to the field plots. The statistical design of this study was a Randomized Complete Block Design with three replications. During the period of full flowering, selected phenological (number of days to flowering), morphological (plant height, mean leaf area, number of black nodules/leaf) and chemical (hypericin and pseudohypericin content) characteristics were assessed. Our observations were that the 'Veperikon' cultivar is very sensitive to soil-borne diseases. All transplanted seedlings were infected by the plant pathogenic fungus *Colletotrichum gloeosporioides* (Penz.), which caused necrosis of the whole plant. Both the 'Gold' cultivar and plants from the wild population persisted despite mild infections with *C. gloeosporioides* and produced flowering shoots at both the first and second years after cultivation. The 'Gold' cultivar was superior to the *Ardabile* population in terms of phenological and morphological characteristics. The average naphthodianthrone contents (% dry weight of tissue) for the wild Iranian population were 0.09(±0.03)% but for the 'Gold' cultivar, 0.65(±0.12)%. These data indicate that selection and directed cultivation of Iranian *H. perforatum* plants can result in plants with improved morphological, phenological and chemical characteristics.

Sm08.252

Content and Chemical Composition of Essential Oil of 'Alecim-Pimenta' in Manaus - Amazonas State, Brazil

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Lippia sidoides Cham. is a large shrub or small tree, typical of the scrub vegetation of the Brazilian Northeast, whose leaves are rich in essential oil content reaching up to 4.5%. This oil is rich in thymol, which has antimicrobial activity against fungi and bacteria. Due to its importance, this species has been cultivated throughout Brazil. In the Amazon it has been developing well to local conditions, producing high biomass and satisfactory growth. The objective of this study was to investigate the essential oil content and its chemical constituents, cultivated under the conditions of Manaus - AM. Plants with a year old, obtained from stems, fertilized every six months with organic fertilizer (cow manure - 2 kg/m²) in the reproductive phase, were cut and taken to the Laboratory of Phytochemistry and Medicinal Plants of Embrapa Western Amazon. After separation of leaves, two samples of 20.0 g were used for determination of moisture, using an oven at 65 °C for three days. Two other samples of 100.0 g each were used for determination of essential oil. The yield of essential oil was expressed on dry weight basis. The yield was 4.36%. The major constituents of the essential oil were thymol - 76.6%, orthocymene - 6.3%, beta-caryophyllene - 5.0%, gamma-terpinene - 2.0%, myrcene - 1.1%, 4-terpineol and Timile-methyl ether, both with 1.0%. Constituents be-

low 1.0% were identified: alpha-tujeno, alpha-pinene, alpha-terpinene, limonene, 1,8-cineole, ipsdienol, umbelulone, alpha-terpineol, alpha-copaene, aromadendrene, ledeno, delta-cadinene and oxide caryophyllene. The content of essential oil and its components showed values close to that found in the conditions of natural occurrence local of the species.

Sm08.253

Biomass Production and Chemical Composition of Essential Oil of *Piper callosum* as Affected by Spacing in Manaus - Amazonas State, Brazil

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The family *Piperaceae* globally presents 12-14 genera and about 1400 to 1950 species, 700 species of the genus *Piper* and 600 species of genus *Peperomia*, distributed in the tropics. Brazil has five genera, and *Piper* and *Peperomia* predominate with 170 and 150 species, respectively. In general, are herbaceous plants or shrubs, with entire leaves, alternate, inflorescence with very small hermaphrodite flowers. The species of the genus *Piper* have as main characteristic, in any organs of plants, strong aroma and sweet and spicy flavor. They offer wide variety of uses as spices, flavorings and medicinal. The objective of this study was to evaluate the biomass of aerial part (dry weight basis), essential oil yield and its components, of *Piper callosum*, affected by different spacings in plants cultivated in Manaus - Amazonas States, Brazil. The experiment was conducted at Embrapa Western Amazon, in Manaus, AM, Brazil. The experimental design was randomized blocks, with four treatments (E1 - 0.5 x 0.5 m; E2 - 0.5 x 1.0 m; E3 - 1.0 x 1.0 m; E4 - 1.0 x 1.5 m) and seven replicates with six plants in useful area. The seedlings were made from cuttings of the stems and were planted in February 2007. In December of that year, they were cut at 10 cm of soil level. Biomass production was inversely proportional to the spatial arrangements, with the greatest biomass production (1,034.93 kg / ha) in the shortest spacing (E1), although no statistical difference was verified between E3 and E4. The same response was observed for the production of essential oil. For the chemical composition, regardless of spacing, it was found that the majority was composed of saffrole (59.1%), followed by beta-pinene (8.3%), alpha-pinene (6.5%). Other constituents found were methyl eugenol (6.3%), 1,8-cineole (4.1%), sabinene (2.4%), gamma-terpinene and elemicine, both with 2.0% and beta-caryophyllene (1.2%).

Sm08.254

The Intercropping Fennel and Bean in Brazilian Semi-Arid Region

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Fennel (*Foeniculum vulgare* Mill.) is a spice and medicinal plant from Mediterranean region. In the Brazilian northeast, this crop is grown by family farmers, without irrigation, under high temperature conditions. A field experiment was conducted in semi-arid of Sergipe state, Brazil, to evaluate the performance of fennel - bean intercropping, under organic management, regarding to the agronomic traits, Area Equivalency Index (AEI) and components of yield. The experiment was conducted in a complete randomized blocks design with six replications and four treatments. The treatments were single crop and intercropping (additive model) of fennel with one variety of cowpea (*Vigna unguiculata*) and two varieties of beans (*Phaseolus vulgaris*), under organic management. Additionally, cowpea and beans were also grown as single crops. Plants of fennel were obtained from seeds sowing in trays, filled with organic substrate. The field

experiment was carried out for 167 days, from June, at the beginning of the rainy season, until the harvest of umbels on January. After harvest, the umbels were dehydrated in drying room, at room temperature (34 °C) and relative humidity kept at 40%. It was determined the dry mass of umbels, absolute and relative yield, AEI and essential oil content. No significant differences were detected as related to yield, which averaged 686 kg·ha⁻¹, but the height and canopy area of the plants were higher on the intercroppings. AEI values confirmed the viability of intercropping fennel and bean. It was also found that the production of dried umbels in all treatments was highest the fourth harvest, when it reached peak and then declined. The incidence of pests (especially aphids) was very low, causing no reduction in production.

Sm08.255

Rosemary (*Rosmarinus officinalis* L.) Essential Oil Composition along an Altitudinal Gradient in the Requena-Utiel Uplands (Western Valencia, Spain)

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Rosemary essential oils have been accepted and recognized as having several therapeutic applications. The diverse composition of the essential oils of different rosemary's ecotypes in a given area may modify their level of activity. This preliminary study attempts to provide a contribution on the relationship between chemical composition and biotypes and/or chemotypes. The chemical composition of the essential oil of spontaneous *Rosmarinus officinalis* is presented for three populations sampled in February and distributed in an altitudinal gradient in the Requena-Utiel uplands of the Valencia region (Eastern Spain). The relationship between bioclimatic indexes and edaphic factors with chemotypes is determined through the application of linear discriminant analysis (LDA) to both ecological and the essential oil data set components. A total of 28 compounds have been identified in the oils. The essential oil composition of the three populations of plants appeared quite different and allows us to identify three different chemotypes. Chemotype eucalyptol (36% eucalyptol, 17% camphor, 13% α -pinene) shows a positive relationship with higher mean annual temperature, higher minimal temperature of the coldest month, and low nutrient content of soil, characteristics of the lowest altitude. At higher altitudes, the chemotype camphor (32% camphor, 24% eucalyptol, 11% α -pinene) seems to be related to better soil nutrient contents and hydric balance in plants and soils, and higher minimal temperature of the coldest month than another chemotype also found at high altitude which characterized by similar proportions of eucalyptol and camphor. It seems that Rosemary plants with an oil composition with camphor contents above 24% resist to freezing and grow at altitudes between 900 and 1200 m.

Sm08.256

In vitro Micropropagation of *Meristotropis xanthoides* Vassilcz

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Glycyrrhiza L. (*Fabaceae*) consists of perennial herbs grow in sandy soils with hard lightness. Three species of the genus grow in Iran, among them *G. glabra* has the widest distribution. It is well known in English as Licorice and the root extract includes flavonoids and Glycyrrhizin are used widely in medicine, food industry, tobacco products and other industries. *Meristotropis* Fisch. & C. A. Mey with only species of *M. xanthoides* Vassilcz is the most closely related genus to *Glycyrrhiza* L. and likely has *Glycyrrhizin*. For weak germination potential, propagation of these plants needs a long time. In addition, germinated seeds always do not produce

uniform and pathogen free clones. Hence, *in vitro* micropropagation of these plants can be helpful for maintaining germplasm, shortening duration of development and accessing uniform and pathogen free clones. A method for *in vitro* micropropagation of *M. xanthoides* was developed. Hypocotyl, cotyledon and stem explants obtained seedlings were cultured on MS media containing different concentrations of NAA and BA. Following optimization of the growth regulator combinations in the media, large numbers of shoots were propagated from a stem explant within 8 weeks. While hypocotyl explants produced shoots at a lower frequency, cotyledons only produced calli with no morphogenetic potential. Excised shoots proliferated at a frequency of 70% within 4-5 weeks in half strength MS medium containing 0.5 mg/l BA.

Sm08.257

Cutting of *Urtica dioica* L. Treated with Butyric Acid and Naphthalene Acetic Acid

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The vegetative propagation of medicinal species has attracted interest from agricultural research, since it is the starting point and a basic tool for any cultivation on a commercial scale. For this purpose a study was set out to analyse the rooting of *Urtica dioica*, treated with Indole Butyric Acid (IBA) and Naphthalene-Acetic Acid NAA (NAA). Hardwood, semi-woody and herbaceous cuttings were taken and treated with growth regulators at a concentration of 1 mg·l⁻¹ by dipping the base of the cuttings for 2 minutes. The cuttings were planted in plastic pots using a substrate of Plantmax[®] for 60 days. Number of roots per cutting, rooting percentage and length of the main root were recorded. The experiment was a 2 x 3 factorial (phytohormone vs. types of cuttings) design with 5 replicates. The hardwood cuttings treated with IBA had the best rooting in relation to herbaceous and these better than the semi-woody. No difference was recorded between hardwood and semi-woody cuttings which rooted entirely with the use of NAA, and higher than for the herbaceous cuttings. The experiment showed that cuttings of *Urtica dioica* root easily, even without hormone treatment, and being more satisfied with the use of hardwood and semi-woody cuttings.

Sm08.258

Establishment of Efficient Callus Induction System of *Astragalus mongholicus* Bge

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Astragalus Radix, the dry root of *Astragalus mongholicus* Bge, has been used for centuries as an important medicine to reinforce vital energy, and to promote the discharge of pus and the growth of new tissue. Since *Astragalus mongholicus* Bge, has a long cultivation cycle, are greatly impacted by the environment production can be unstable and growers can hardly satisfy the market demand. The establishment of suspension cell lines is the best solution to address this problem. This study uses MS medium to study the different concentrations and ratios of hormones, different explants and light on the impact the formation of callus of *Astragalus mongholicus* Bge, the results showed that in MS medium with 1.0 mg / L 2,4-D added the rate of callus induction was 50.0%. 2,4-D had a much better effect on induction than 1.0 mg / L of NAA (the rate was 26.32%), and callus grown were in good condition. The best combination of imposed concentration was 2.0 mg / L 2,4-D +1.0 mg / L 6-BA, the rate of callus induction was as high as 93.33%, and time to callus growth callus was as early as 2-3 days. The order from high to low of rates of induction of different explants was cotyledons, hypocotyl and radicle, but cotyledons' growth status was not as good as hypocotyl growth status. Compared with the dark culture, light culture was more conducive to callus induction and growth. The rates of hypocotyls, cotyledons and radicle induction of were 83.33%, 93.33% and 46.67%, while for the dark the rates were 73.33%, 90.0 % and 26.67%, respectively. This study will provide the foundation of establishment of suspension cell lines of *Astragalus mongholicus* Bge.

Sm08.259

Toxicity of Volatile Phase of Essential Oils from Aromatic and Medicinal Plants to Apple Fruit Pathogens *in vitro*

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Postharvest losses, caused by fungal diseases, are the major factor limiting the storage period and market life of apples. Currently, the control of postharvest decay is mainly based on the use of synthetic fungicides during growing season. However, the use of fungicides is restricted due to health concerns and consumers' demand for less chemical residues on fresh produce. Application of substances of natural origin could be a convenient solution, safe for both human health and the environment. Essential oils from aromatic and medicinal plants are well known for their antimicrobial activity. Therefore, the objective of our study was to investigate the effect of volatile phase some essential oils on the growth of certain apple fruit pathogens: *Monillinia* sp.; *Botrytis cinerea*, *Colletotrichum* sp.; *Fusarium avenaceum*, and *Alternaria* sp. In order to determine fungicidal activity of the oils, the cultures, grown on artificial media in Petri dishes, were exposed to volatile phase of the oils. The exposure of the pathogen to the investigated oils lasted seven days and was followed by ventilation in a laminar flow hood. A culture of the pathogen grown without any treatment was used as a control. The growth rate of the tested microorganisms was partially or completely inhibited by the oil applied at concentrations 0.04 - 0.65 µl/ml air. Thyme essential oil showed fungicidal effect on all the tested pathogens at the concentration of 0.16 µl/ml air. The effect of cinnamon oil was lethal to *Monillinia* sp. *Colletotrichum* sp. at concentrations 0.04 and 0.08 µl/ml air, respectively. Parsley essential oil showed the lowest antifungal activity with minimal lethal concentration higher than 0.65 µl/ml air. These results indicate that some of the tested essential oils could be used for the control of investigated pathogens.

Sm08.260

Micropropagation of *Lavandula multifida* L. from Field-Growing Adult Plants

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The selection of native *Lavandula* species and their economic exploitation have increased in the last few years. Micropropagation techniques have been used as an alternative for vegetative propagation allowing the multiplication of selected genotypes. For the first time an efficient protocol has been established for *in vitro* propagation of field-growing *L. multifida*. Axillary buds from adult plants were established in Murashige and Skoog (MS) medium supplemented with 10 mg/L ascorbic acid. Several concentrations of the cytokinins benzyladenine (BA, 0.0; 0.1; 0.2; 0.5 and 1.0 mg/L) and zeatin (0.0; 0.1; 0.2; 0.5 and 1.0 mg/L) were tested on shoot proliferation and two concentrations of indol-3-butyric acid (IBA, 0.5 and 1.0 mg/L) were used to analyze rooting. The highest multiplication rate (6.1 shoots per explant) and the highest number of nodes per shoot (5.9) were obtained using MS with 1.0 mg/L zeatin. However, longer shoots (1.5 cm) were formed using MS with 1.0 mg/L BA. Shoots exhibited a normal development but *callus* usually developed at the cut ends of the explants in all media supplemented with cytokinins. Shoots were then separated from the *callus* and transferred to MS medium. After a month in culture 41.75% of the shoots rooted spontaneously. The remaining shoots were cultured on MS supplemented with IBA. No significant differences were found concerning the number and length of roots formed on media with different IBA concentrations. Rooted plantlets were successfully transferred to soil, exhibiting a normal development with high degree of homogeneity and no evidences of somaclonal variation. Flowering was observed during the acclimatization phase of the micropropagated plants. Micropropagation through axillary

shoot proliferation showed to be a reliable method for the rapid multiplication of *L. multifida* allowing plant conservation without damage of the natural resources, which are very limited in Portugal.

Sm08.261

Evaluation of Total Antioxidant Production in Seed and *in vitro* Populations of *Taraxacum mongolica* and *T. officinale*

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Various members of the genus *Taraxacum* are widely used in traditional medicine to treat complaints including hepatic disease, various cancers, inflammation and urinary problems. Scientific examination of major species from the genus has identified a range of phytochemical compounds with characteristics that include anti-inflammatory, anti-carcinogenic, analgesic and antioxidant activity¹. To investigate the potential for the production of desirable, active compounds such as antioxidants from commercial field cultivation, a seed population of *T. mongolica* collected in the wild in the Chang Bai mountain region of Northeastern China was compared with a population of *Taraxacum officinale*, native to E. Denmark for total antioxidant activity². Additionally, a protocol for the rapid micropropagation of *Taraxacum mongolica* was developed, with markedly restricted *callus* formation and precocious shoot development, to provide clonal material for subsequent field propagation likely to express minimal somaclonal variation. Total antioxidant levels in mature leaves of the two *Taraxacum* species were broadly comparable, with median values ranging from 27.9 to 32.9 Trolox equivalents (expressed as µMTrolox/g. fw). However, when a population of micropropagated plants of *T. mongolica* (each plant from a different parent) was examined, the median values for antioxidant, and the range, within the population, were significantly diminished. This suggests that such stresses as may be experienced *in vitro* induce a lower antioxidant response than those experienced in the natural environment and effective that field production might require the application of controlled stress to maximize antioxidant yield as a product.

Sm08.262

Micropropagation of Chinese Foxglove (*Rehmannia angulata*) Using a Tissue Culture Medium Based on Formula β for Mineral Composition

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Chinese foxglove (*Rehmannia angulata*) may have important potential in pharmacology, as it is closely related to the highly valued traditional Chinese medicinal plant *Rehmannia glutinosa*. Previous studies on micropropagation of hemp (*Cannabis sativa* L.) have shown that the inclusion in tissue culture medium of Formula β, a newly developed basal salt mixture composed of 2 parts (A+B), has given a significantly higher shoot multiplication rate than on MS based medium. In the present study, the effects of Formula β on micropropagation of Chinese foxglove (*Rehmannia angulata*) have been investigated in the shoot/root multiplication and acclimation stages. Formula β based medium was significantly more effective than MS based medium after eight week of *in vitro* culture to induce new shoots by regenerating approximately twice as many shoots per explant, while the root multiplication rate was nearly three times higher. Moreover, the highest mean number of leaves produced *ex vitro* was observed at the end of the acclimation stage on plants that were previously *in vitro* cultured on Formula β based medium. Hence, it proved that the tissue culture medium based on Formula β could efficiently im-

prove the micropropagation rate of Chinese foxglove (*Rehmannia angulata*). This will be useful to produce large quantities of plant materials for further biochemical characterization, phylogenetic analysis, pharmacological research and pharmaceutical production.

Sm08.263

Radioprotective Activity of Some Medicinal Plants Extracts

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Exposure to radiation has significantly increased during the past hundred years with the development and use of x-rays and radioisotopes in medicine and through environmental pollution from nuclear weapons and power plants. Because radiation can induce mutagenic changes, interfere with the immune system, and lead to the development of cancers, agents that could protect the body from radiation effects would be of great benefit. In this study, aqueous extracts of saffron (*Crocus sativus*), yarrow (*Achillea nobilis*), and scholar tree (*Sophora japonica*) reduced mutagenic effects of γ -radiation on albino Wistar rats. The most effective radioprotection and anti-mutagenic activity were demonstrated by the saffron extract, which reduced frequency of chromosomal aberrations in bone marrow cells (35.7% at 3 Gy; 17.1% at 5 Gy) and sperm cells (26.9% at 3 Gy; 16.4% at 5 Gy) and restored cell division. Radiation was applied in a gamma-ionization chamber at the Rk-hund-20000 facility located at the Institute of Radiation Problems in Baku using an average power of 1252 rad-sec⁻¹.

Sm08.264

Antioxidant Activity of *Lavandula latifolia*, *Salvia lavandulifolia* and *Thymus mastichina* Collected in Spain

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Industry has been using antioxidants and their positive effects in order to maintain food quality and to prolong its long life. Some of these compounds are synthetic; however, it might be highly desirable to find out natural antioxidants. Aromatic plants help to improve organoleptic qualities of food products and also contribute to their preservation. Owing to this, the antioxidant activity of some populations of *Lavandula latifolia* (Ll), *Salvia lavandulifolia* (Sl) and *Thymus mastichina* (Tm) have been evaluated. Ten populations of Tm and 12 of each Sl and Ll have been collected at flowering stage during the summer of 2009 around Castilla y León (the Central Region of Spain). The vegetal material was dried in dark and room temperature conditions before hydrodistillation. Dry plant material and dry residues after hydrodistillation were used for analysis. Total phenolic content and antioxidant activity were determined. Two methods have been used to determine the antioxidant activity: 1) Free-radical scavenging (DPPH), and 2) Reducing power assay. The results showed that Tm had the higher content in essential oil, although there was high variability among the populations, indicating the selection will be possible in the three species. Total phenolic content and antioxidant activity of dry plant material were higher than hydrodistilled dry material, showing that a considerable portion of antioxidants was retained in the remaining hydrodistillation-aqueous water and the essential oil. Total phenolic content and antioxidant activity of dry plant material were also higher in Tm. In the case of hydrodistilled dry material, there were no differences between Tm and Sl, both species had higher phenolic content and antioxidant activity than Ll. Although there have been variability among the populations and selection would be possible, Tm is postulated as the most interesting species from an antioxidant activity point of view.

Sm08.265

Volatiles from *Coriandrum sativum*: Comparison of *in vitro* and *ex vitro* Grown Plants

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Coriandrum sativum (coriander) is commonly used, raw or cooked, in Portuguese gastronomy. Coriander is also used in traditional medicine as a carminative and as a digestive aid. The fruits essential oil is used in food flavouring and in perfumery and is also responsible for the digestive and stimulant effect as well as for fungicidal and bactericidal activity [1]. *In vitro* *C. sativum* plants were established by micropropagation in MS medium from *in vitro* grown 3 weeks old coriander seedlings. Six months after multiplication, some plants produced anthocyanins and maintained this phenotype for, at least, two years under *in vitro* micropropagation. In the present work, the volatiles of fruits and of *ex vitro* and *in vitro* *C. sativum* plants, with (A) and without (B) anthocyanin production, were studied. The volatiles were isolated by hydrodistillation and analyzed by GC and GC-MS. The fruits volatiles were dominated by linalool (82%), with γ -terpinene (4%), camphor (3%) and geraniol (3%) as other major compounds. Linalool was present in the volatile fractions of A and B *in vitro* and of *ex vitro* grown plants in small relative amounts (0.1%, 0.1% and 0.3%, respectively). Dodecanal (17%), n tetradecanol (15%) and n nonane (7%) were the dominant compounds in the *ex vitro* plants volatiles. β Phellandrene (37%, 45% in A and B, respectively), terpinolene (both 9%), β -sesquiphellandrene (4%, 6% in A and B, respectively) and α phellandrene (2%, 3% in A and B, respectively) were the major compounds in of A and B *in vitro* grown plants. Despite the anthocyanin production in A *in vitro* grown plants, the volatile profile was quantitative and qualitatively very similar to that of B *in vitro* grown plants.

Sm08.266

In vitro Establishment of Aromatic Geranium from Brazil

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The aim of this work was to realize *in vitro* establishment of aromatic *Pelargonium graveolens* L'HÉR ex Ait. from Brazil. The completely randomized design was used. A 4 x 4 x 2 factorial scheme was utilized in the first assay, testing four concentrations of sodium hypochlorite (1.0; 1.5; 2.0 and 2.5%), four immersion times (8, 10, 12 and 14 minutes) and with and without pulverization with fungicide and antibiotic of the mother plants before removing explants. In the second assay a 4 x 4 x 2 factorial scheme was utilized, testing four concentrations of mercury chloride (0.06; 0.08; 0.10 and 0.12%), four immersion times (8, 10, 12 and 14 minutes) and with and without pulverization with fungicide and antibiotic of the mother plants before removing explants. For the third assay a 4 x 2 factorial scheme was utilized, testing four concentrations of salts of the MS medium (25%; 50%; 75% and 100%) and two types of explants (nodal and leaves). The evaluated variables were regeneration (%), contamination (%), number of shoots per explants, dry weight of the aerial part (mg). Sodium hypochlorite at 1.2% for 12 minutes was efficient for disinfection of geranium explants. The concentrations of 0.09 and 0.08% of mercury chloride for 12 and 14 minutes, respectively, presented best results for contamination control. The use of leaf explants in MS medium containing 39.8% of its salts allowed major regeneration rates of geranium.

Sm08.267

Tissue and Cell Cultures of *Hypericum undulatum* for the Production of Acetylcholinesterase Inhibitors

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Hypericum undulatum Willd. (Guttiferae), general name wavy St. John's wort, is a medicinal plant. It is traditionally used for renal antispasmodic, hepatic protector, and the treatment of migraine, bladder and gall bladder ailments and intestinal-inflammatory. Alzheimer's disease (AD) is frequent in elderly people, being the leading cause of dementia among older people. An estimated 10% of the world's population over the age of 65 years is afflicted by AD. Acetylcholinesterase inhibitors (AChEI) are currently the best available pharmacotherapy for AD patients. Presently, treating the symptoms of AD can only delay the progress of the disease. In addition, all the present medicines for AD have side effects. Therefore, it is of importance to screen for more powerful drugs from natural products to treat AD with fewer side effects. A recent work has demonstrated that *H. undulatum* plant has the AChEI activity. In this work, cell suspension cultures of *H. undulatum* were established for the production of the AChEI. Seeds were sterilized and aseptically germinated on MS medium solidified with agar without plant growth regulator. The germinated plants were maintained and used for *callus* induction. The best medium for *callus* induction and growth was MS plus 1 mg/l 2,4-D, 1 mg/l NAA and 0.2 mg/l 6-BA. Dispersed white *calli* were transferred to the same medium but without agar to establish cell suspension cultures. The suspension cultured cells turned dark and formed big cell blocks after subculture more than two months. Different cultivation parameters were tested to optimize the cell growth for a continuous culture. Kinetics of cell growth and sugar consumption was analyzed. The AChEI activity of the plant cell extract was determined by capillary electrophoresis. The results shown that upon metabolic regulation by elicitors the suspension cultured plant cells had a higher AChEI activity than that of the plants.

Sm08.268

Cloning of Pharmaceutical *Cannabis* through an Aeroponic Propagation System

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Cannabis sativa L. is an important pharmaceutical species because it is the only source for a whole series of chemically diverse bioactive compounds that are currently under intensive investigation. Cuttings of pistillate plants is the preferred propagation material for the pharmaceutical production to ensure continuous chemotype correspondence of clonal progenies. Aeroponic propagation gave satisfactory results on cloning of many economic important plant species and for that reason the aim of this study is to evaluate the feasibility of its use in the cloning phasis of *Cannabis* pharmaceutical production. Two experiments were conducted to evaluate the rooting capacity of three different stock plants ('mostly *sativa*', '*sativa/indica* hybrid', 'mostly *indica*') and the rooting capacity of cuttings taken from three different positions (top, middle, bottom) of the stock plants used. Stock plants were selected from recreational strains on the basis of Δ9-THC yield per crop area unit and kept in vegetative stage in artificial growing conditions. The aeroponic propagation system resulted easy to use and efficient to observe root initiation as the cuttings remain suspended in the air. Significant differences on rooting capacity were found between the different stock plants used and the different positions from where the cuttings were taken. The highest percentage of rooted cuttings was obtained from a 'mostly

sativa' biotype (80%), followed by a 'mostly *indica*' biotype (70%), and the lowest value was obtained from a 'hybrid *sativa/indica*' (67%). Direct correlation was found between the percentages of rooted cuttings and the average of root lengths after 14 days from the edge of cuttings. Cuttings taken from the bottom position of stock plants had the highest rooting capacity. Cuttings were ready for transplantation in 14 days without the application of plant growth regulators. Despite the satisfactory results obtained, further research is needed to optimize the technique.

Sm08.269

In vitro Culture of *Coriandrum sativum*

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Coriander (*Coriandrum sativum* L.) is a plant from the *Umbellifera* family. In Portugal, the use of coriander plants in gastronomy is very common, like in all the countries in the Mediterranean area. This plant species has also several other applications than as an aromatic plant, such as medicinal, being recommended for dyspeptic complaints, loss of appetite, convulsion, insomnia and anxiety. Moreover, the essential oils and various extracts from coriander have been shown to possess antibacterial, antioxidant, antidiabetic, anticancerous and antimutagenic activities among others, it has also been used as a flavoring agent in food products, perfumes and cosmetics. To study the potential use of this plant all over the year it is necessary to establish an *in vitro* system production and to evaluate the better conditions for its growth. *In vitro* coriander cultures were started from seeds of *Coriandrum sativum* from a commercial origin. Seeds were inoculated in a MS medium containing different concentration of IBA and BAP. After 6 months of *in vitro* culture, the plants were separated in two lots named lot A and B differentiated by their pigmentation (clones with differentiation in flavonoids accumulation), being lot B the less pigmented and lot A the one who presented an higher purple coloration, under the same *in vitro* growth conditions (nutrition, temperature and light). The growth rates of both lots were determined through fresh and dried weights and evaluating how pigmentation affects these parameters. The medium with better growth rates was MS with 0.1mg/L IBA and 0.1mg/L BAP; The B lot grew better but have the stationary phase after 3 weeks while the A lot was still growing after 4 weeks but grew slower comparing wit lot B. These results will be used in further studies concerning the essential oils production in each lot.

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Determination of Antibacterial and Antiradical Activity of *Origanum vulgare* Clones Grown in Latvia

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Origanum vulgare is widely used in pharmacies and folk medicine. *Origanum vulgare* clones grown in Latvia differ from their phytochemical content. The aim of the study was to determine the antimicrobial and antiradical activity of these clones. Antibacterial and antiradical activity of ethanol extracts prepared from 10 *Origanum vulgare* clones grown in Latvia was screened. The antibacterial activity was assessed against bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Bacillus cereus*). A sensitivity tests were performed in the liquid nutrient media for bacteria. Plant ethanol extracts from leaves and flowers (25-40 g of fresh matter per L) were added to the growth media. Extract and media proportion was 1:20. Microorganisms growth were detected spectrophotometrically at wavelength 550 nm after 24 and 72 hours of incubation at 28 °C. Plant extract antiradical activity was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH). *Origanum vulgare* leaves and flowers showed different activity. The antimicrobial and antiradical activity depends on *O. vulgare* clone and sampling time. Different clones showed unlike activity on used microorganisms and it depends on oregano chemical content.

Sm08.271**Experiment with Family Farmers on the Production of Fennel (*Foeniculum vulgare* Mill) in Ecologic Bases in Sergipe's Semi-Arid Region****Curado, F. F.; Nunes, M. U. C.; Carvalho, L. M.; Oliveira, I. R.; Rodrigues, R. F. A.**EMBRAPA TABULEIROS COSTEIROS, BEIRA MAR AVENUE, 3250, JULY 13, 49025-040, ARACAJU, SERGIPE, BRAZIL

The frequent production of fennel in ever-smaller areas without the proper reposi-
tion of nutrients is favoring the impoverishment of the soil, such as it was observed
in Simão Dias Township, in the state of Sergipe, Brazil. In addition to this, the
agro-ecosystems' imbalance due to the advance of corn monoculture has favored for
an increase of plant louse infestation. The action of this aphid takes place during the
inflorescence of the plant, affecting fruit and seed production and resulting in great
financial loss to farmers. For this reason, Embrapa Tabuleiros Costeiros and EM-
DAGRO - Sergipe's Agronomy and Livestock Development Agency, have joined
forces in order to develop a research on the socio-economic and environmental char-
acterization of the reality of small family-owned rural fennel producing properties.
The study concentrated on the traditional production of fennel and was developed
with the participation of approximately 70 families in three rural communities. This
allowed for an exchange of technical knowledge related to agro-ecology. The re-
search took place in two 'UEs' - Experimental Units, where informal trials reflected
the convergence between the investigative concerns of the farmers and the research-
ers. These trials were conducted over one productive cycle of fennel of approxi-
mately 210 days, under local edafoclimatic conditions. The results demonstrated
the pivotal role of the agro-ecological production of this aromatic plant, allowing
for new perspectives on plant louse control as well as of soil and water management
to be considered in future studies. The indirect sowing was positively evaluated by
the farmers since it allowed for early fennel harvest expectancy and consequently
the securing of better prices. Moreover, the experiment with farmers represented an
important methodological learning process to be enlarged in any future research op-
portunity that is aimed at familiar agriculture in Sergipe's semi-arid region.